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A PICKY PALETTE? THE HOST PLANT SELECTION OF AN ENDANGERED BEETLE

A Thesis Presented to

The Faculty of the Department of Environmental Studies

San Jose State University

In Partial Fulfillment

of the Requirements for the

Degree Master of Science

by

Kirsten E. Hill

May 2006

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APPROVED FOR THE UNIVERSITY

Thea I. Williamson

APPROVED FOR THE DEPARTMENT OF ENVIRONMENTAL STUDIES

DR. RACHEL O'MALLEY, ASSOCIATE PROFESSOR AND CHAIR OF THE DEPARTMENT OF ENVIRONMENTAL STUDIES, SAN JOSE STATE UNIVERSITY

DR. JEFFREY HONDA, ASSOCIATE PROFESSOR, DEPARTMENT OF BIOLOGICALSCIENCES, SAN JOSE STATE UNIVERSITY

Richard A. Arnold

DR. RICHARD ARNOLD, ENTOMOLOGICAL CONSULTING SERVICES LTD., PLEASANT HILL, CALIFORNIA

ABSTRACT

A PICKY PALETTE? HOST PLANT SELECTION OF AN ENDANGERED BEETLE

by Kirsten Hill

Little is known about the endangered Mount Hermon June Beetle (*Polyphylla barbata)* (MHJB) found only within Zayante soils region of Santa Cruz County, California. For the reason that research uncovering host plants of the species would benefit habitat management for the region, the study objective was to identify the MHJB's host plant selection and habitat association. Mating behavior and microsite plant assemblages in female burrow sites, male flight regions and adjacent sites were analyzed between June 2004 and September 2005. Larval frass was collected from specimens found in female burrows. Larval species was confirmed by DNA analysis. Microsite flora analysis identified a variety of flora in the regions of MHJB association. Significant differences were found between regions where the MHJB did and did not occur for *Chorizanthe pungens var. hartwegiana* and *Pinus ponderosa*. Frass analyses identified angiosperm and pteridophyta divisions, and fungi demonstrating that MHJB larvae are not specialist feeders.

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INTRODUCTION

In recent years, an emerging focus of science has been to understand the global loss of biodiversity. Scientists seek to understand the biology of species before they disappear. The need to understand and conserve invertebrates has increased substantially over the last few years as we have come to recognize they also are disappearing as rapidly as other plant and vertebrate species and maybe even more so. Insects can often act as indicators of an ecosystem's health as well as play roles as keystone species. Fossorial and other ground dwelling insects in particular, may play an important role in understanding the general health of an ecosystem. Ecosystem functions, such as nutrient recycling are often done by ground dwelling and specialist insects that are not well understood as it is difficult to understand underground life cycles. An example of this lack of understanding of fossorial insects can be seen in the case of the Mount Hermon June Beetle (MHJB), *Polyphylla barbata* (Cazier). Although we know enough about the MHJB to recognize that it is endangered, we do not know enough about its food source and environmental requirements to provide it with adequate protection and to identify appropriate management practices to benefit it. To date, very little research has been conducted regarding most *Polyphylla* species food sources; research thus far has been focused on the adaptive radiation of various species in the genus. It is important that we learn more about the MHJB and insects in general in order to help protect and provide for earth's endangered ecosystems.

The MHJB is found only within the Zayante Sandhills of the Santa Cruz Mountains located within the San Francisco Bay Area, a recognized biological diversity hot spot in which 50% of the species of arthropods listed by the U.S. government as endangered occurs (Connor, et al. 2003). The Zayante Sandhills cover no more than three percent of Santa Cruz County, support numerous rare and native plant species, and are considered to be "biological islands," distinctive areas where certain species thrive (U.S Fish and Wildlife Service, 1997). The several rare and endangered inhabitants of this area continue to face considerable threats from human activities including urban development, recreational activities and sand mining. The Zayante soil is also prone to erosion, compaction, and the vegetation is prone to fire, all of which may disturb the beetle's life cycle. It has been estimated that prior to human disturbance, the Zayante Sandhills habitat covered 6,265 acres (Lee, 1994). In total, more than 40% of the Zayante Sandhills habitat is estimated to have been lost to or altered by human activity (U.S. Fish and Wildlife Service, 1997).

Past observations of the MHJB have led many to believe it is specialist feeder, but without any definitive research, it is difficult ascertain this species host plant range. An endangered species cannot adequately be protected until its food source has been identified and protected as part of the species habitat. Much of the information available regarding the MHJB feeding habitats is anecdotal and very little information is available. To date, no scientists have attempted to confirm potential food sources of the MHJB (see Arnold, 1999; Hazeltine, 1993; U.S. Fish and Wildlife, 1997). Because the MHJB is found within such a confined region and within endemic plant communities, it is likely to be a host plant specialist making use of one or at most, a few host plants. It is also likely the MHJB has coevolved in tightly woven interactions with some of the highly adapted, endemic Sandhills flora.

Importance of Research

Research uncovering the potential host plant(s) of the MHJB will guide management practices setting aside potential habitat for this species and assist policy-makers in providing the best protection for the Sandhills. The goal of putting a species on the endangered species list is to protect and promote the recovery of that species. Understanding the MHJB's host plant(s) choice is important for protecting and promoting its recovery. On a broader level, the

MHJB is indicative of the health of the unique ecosystem in which it exists. As with any species within an ecosystem, it can be viewed as part of the intricate web of all plants and animals within the Sandhills environment. The endangerment or extinction of any one species within an ecosystem indicates to us a warning of greater danger. If signs are left unheeded it is possible that such a fragile ecosystem could face rapid destruction and collapse.

RELATED LITERATURE

Natural History of the Mount Hermon June Beetle

The MHJB was initially described in 1938 by Cazier. In 1940, through Cazier's work, the beetle was recognized as a distinct scarabaeidae species in the genus *Polyphylla*. Since Cazier's first descriptions of the MHJB, it has been studied by a scattering of scientists, who have uncovered information about its general habitat, male flight season, flightless female, and root-feeding, fossorial larvae (see Hazeltine 1993, Arnold 1999, 2004; Russell 1994). In recognition of its limited distribution, the MHJB was listed as an endangered species in 1997 (U.S. Fish and Wildlife Service, 1997).

The MHJB is a small dark brown scarab beetle with fragmented and discontinuous white stripes of scales on its elytra (Figure 1). It is distinguished from three other *Polyphylla* species that occur within regions of Ben Lomond, Felton, Mount Hermon, and ScottsValley by the presence of relatively dense, long erect hairs scattered randomly over the elytra and short erect hairs on the pygidium (last abdominal segment) (Young, 1967,1988). The males are characterized as smaller than females as males are typically 20 mm by 9.7 mm, while females are 22 mm by 12 mm. The small mouthparts and limited flight period of the adult MHJBs suggest that they do not feed as adults (Hazeltine, 1993). In an attempt to document the MHJB's food sources, Hazeltine (1993) reared MHJB larvae on a mixture of roots; he was unable to complete his research as the larvae apparently succumbed to fungal infection (Hazeltine, 1994). However, it is known that larval food plants of other *Polyphylla* species include conifers, shrubs, herbs, grasses (Young, 1988; VanSteenwyk and Rough, 1989)

Figure 1: The Mount Hermon June Beetle (male)[1]

[1] Photograph used with permission from Jodi McGraw

During the MHJB's flight season that occurs from May to August, males emerge at dusk from the soil and underneath shrubs and herbaceous plants. Their wings make a distinctive crackling noise as they fly up through the vegetation. They are often found flying low to the ground seeking the source of female pheromones and swarming in an area of a female burrow (Arnold, 1999). Once they are located, females are often the source of competition among several males. The successful male copulates with the female for 2-10 minutes as the female burrows into the soil (personal observation). Male flight activity may be limited by temperature, wind, and may also be affected by cloud cover.

Habitat

Like many *Polyphylla* species, the MHJB has a very narrow distribution and prefers sand, grass and conifer associations similar to those found in the sections of the Zayante Sandhills (Borror et al. 1976). The Zayante soils where the MHJB is found covers about 3% of Santa Cruz County (Lee, 1994). The soil occurs in fragments that are nutrient poor, of little agriculture value, and are considered "excessively drained" (U.S.D.A. Soil Conservation Sciences, 1980). They are derived from loosely consolidated Micocene marine sand deposits known as the Santa Margarita formation (Marangio, 1985). The distinctive character of the soil has given rise to a microclimate that is warmer and drier than surrounding regions that typically support redwood forest flora (Griffin, 1964).

Many plants that grow in this soil have become uniquely adapted and all species that are able to survive in this soil are well adapted to drought. The ecosystem is characterized with several endemic flora and fauna and disjunct populations of species, including the ponderosa pine (*Pinus ponderosa*) (Griffin, 1964). The MHJB's habitat includes areas of this region described as ponderosa pine chaparral habitat that are open and sparsely vegetated areas; dense ponderosa pine forest, and northern maritime chaparral (Arnold, 1999, 2004; Hazeltine, 1993; Russell, 1994). Vegetation in these areas includes bracken fern (*Pteridium aqulinum),* monkey flower (*Mimulus sp.),* oak (*Quercus sp.),* silver leaf manzanita (*Arctostaphlos silvicolai),* grasses, and small annual herbs (Arnold, 2004; Hazeltine, 1993; U.S. Fish and Wildlife Service, 1997). Thus far, the few scientists who have observed the MHJBs flight patterns and burrow locations believe that it may have one or a few food sources, including the roots of ponderosa pine, grasses, monkey flower, oak, and fern (see (Arnold, 1999; Hazeltine, 1993; Russell, 1994).

Quail Hollow Quarry Habitat Conservation Plan

The Habitat Conservation and Recovery Plan (U.S. Fish and Wildlife Service, 1997) for the MHJB species suggests that the beetle may recover from its endangered status if its habitat is protected from sand mining, urban development, and recreational activities. In 1997, Granite Rock Corporation (GRC) applied for an incidental take permit from the Fish and Wildlife Service requesting permission to take the MHJB and three other federally endangered species including the Ben Lomond wallflower (*Erysimum teretifolium),* the Ben Lomond spineflower (*Chorizanthe pungens var. hartwegiana)* and the Zayante Band-Winged Grasshopper (*Trimerotropis infantilis)* within its sand mine (Quail Hollow Quarry). In support of this permit application; GRC prepared a Habitat Conservation Plan (HCP) that provided for conservation and long-term management of four listed species located at Quail Hollow Quarry (QHQ). The Fish and Wildlife permit allows incidental take of species in the "current mining area" and the "future mining area" (FMA) of QHQ (Thomas Reid Associates, 1998).

The HCP limits the mining in the FMA to months other than June, July and August to minimize disruptions in sensitive habitat areas during the height of insect breeding seasons. Other measures taken to preserve habitat in the FMA include removal of nonnative invasive plant species, restricted recreational use (to exclude human pets) by the use of educational signs and erosion and runoff prevention. The HCP also requires the County of Santa Cruz to report annually to the U.S. Fish and Wildlife Service and the California Department of Fish and Game for compliance and mitigation monitoring. For the MHJB, this includes relative abundance surveys to be conducted every other year during the adult flight season of the male June beetle in late June. These monitoring surveys have been performed since 1999 (Arnold, 1999; 2000).

Host Plant Theory

Host plant selection by insects has been the subject of numerous ecological studies (see Scriber, 2002 for a review). Insect diet breadth has also been the focus of many theory driven studies (Futuyma, 2000; Nosil, 2002; Scriber, 2002; West & Cunningham, 2002).

Phytophagous (plant feeding) insects generally fall into one of three host selection categories: 1) Polyphages (generalists) are insects that feed on many different plant groups, 2) Oligophages are insects that feed on a few different plant taxa (e.g. monarch butterfly caterpillars feed on various types of milkweed), and 3) Monophages (specialists) are insects that feed on only one plant taxon. Factors for generalization include rare or unpredictable habitats, difficulties meeting nutritional requirements using a single food source, and greater resource availability. Factors for specialization include competition for resources, resistance to predators, high costs of information processing, more facilitative mate-finding and low costs to searching for suitable habitat (Nosil, 2002; West & Cunningham, 2002).

There are a number of factors that are thought to promote specialization in insects. Narrowed or specialized host selection may be a tactic that can help an insect make best use of a particular plant to maximize defensive behavior against predators. Specialized host selection may also be helpful in defense against predators by incorporating the plants' secondary compounds into the insects' own defense mechanisms (Bernays, 1998a). A leader in the field of insect-plant interaction research, Bernay's studies have addressed issues such as insect neural limitations, the effect of neural limitations on the feeding behavior of insect specialists vs. insect generalists, and the evolution of insect morphology in relation to plants (see Bernays, 1991, 1998a, 1998b, 2001). Bernays (2001) posits that neural limitations of insects may be a primary factor in insects' interactions and coevolution with plants. Insects may be more likely to be specialists because of their limited neural capacity. Choosing a host plant with both optimum quality and suitable location may be problematic for a generalist insect (Bernays, 1998a, 2001). A specialist insect may not be distracted by competing sensory information regarding several similarly attractive hosts. Bernays argues that specialist insects with a more limited sensory capacity may have an evolutionary advantage over generalists in that they have more fixed responses and perhaps faster reaction times given a broad selection of plants from which to choose (Bernays, 1998b).

Generalist insects may have an advantage over specialists in that they are often completely opportunistic, eating largely what is most commonly available. Generalists make use of several different host plants when the nutrient availability is low or when ingested plant toxins from one food source need to be diluted (Singer, Bernays, & Carriere, 2002). Insect generalists are able to complete their life cycle on several species of host plants (Tikkanen, Niemela, & Keranen, 2000). However, food quality is of utmost importance for generalist insects and their life cycle may be greatly effected by the quality of chosen host plants. They are most limited in host plant selection by the presence of plant secondary compounds or deterrents (Renwick, 2001).

Host plant theory posits that among all types of insects, related species of insects commonly are specialized to feed on related plants (Bernays, 1998a; Ehrlich & Raven, 1964; Futuyma, 2000; Scriber, 2002). Futuyma (2000) found that although many newly formed lineages of beetle species have shifted from ancestral hosts to other plant lineages, most shifts are to plants that are within the same tribe. Insects may have genetic variation for characteristics required to shift to some plant species, but lack genetic variation to shift to other plants and they are more likely to shift to certain potential novel hosts over others as they increase their range and face new potential food sources (Futuyma, 1995, 2000). Furthermore, the restricted diet of many insect groups suggests that insects may be genetically constrained.

Most *Polyphylla* beetle larvae are generalist feeders (Russell, 1994; Young, 1988). Some of these species are pests of fruit and nut orchards, conifer farms, and turf grasses (see Bryant, 2002 and Van Steenwyk, 1989). Based on our current knowledge of the MHJB and host plant theory, it is likely that the MHJB is a specialist for the following reasons: 1) the species does not disperse beyond the Zayante soils, 2) the female MHJB is flightless and 3) it faces potential competition within its range for food from three species of the same genus. It is also likely that the MHJB feeds on plants or a plant related to those on which related beetle species are found.

Coevolution Theory

Coevolution theory is based on the premise that species evolve as a result of interactions with other species. By definition, coevolution both requires and produces some degree of specialization within an ecosystem, producing reciprocal genetic responses between species (Thompson, 1994). The adaptive radiation and diversification of insects and plants has long been considered a consequence of coevolution between the two, leading herbivorous insects to colonize new adaptive zones and further evolutionary divergence in their lineages (Becerra, 2003). Plants experiencing herbivory may evolve defenses against herbivore attacks by making use of chemical or physical deterrents. Insect herbivores may then evolve in response to these deterrents with improved digestion and detoxification capacities. Thus, both species evolve in relation to one another leading to plant-herbivore coevolution (Loeuille, Loreau, & Ferriere, 2002). Species can coevolve slowly (over 1000s of years) or quickly (over decades), based on the intensity of the selection pressures within their ecosystem. Important for this study is the finding that animal adaptation that leads toward specialization constrains the potential genetic evolution within species but increases the potential for coevolution among species (Thompson, 1999).

Although *Polyphylla* beetle species are found worldwide, distribution of the genus is typically fragmented. Many of the species are found in association with unique and isolated sandy environment refugia. In total, thirty-one species are found in America, north of Mexico (Arnett, 2001). From last count, 30% of these species are broadly distributed (found in 3 or more states), 18% percent of species are narrowly distributed (found in two or three states) and the remaining 54% are endemic to one or two localities (Young, 1988). Many isolated *Polyphylla* species, including the MHJB are dimorphic, with a flightless female. Young (1988) noted in his *Monograph of the Genus Polyphylla* that the accumulation of dissimilarity in these scarabs is a fairly rapid process in isolated inbreeding populations restricted to one sand dune complex or one mountain top. Further, Young (1988) stated, it is encumbant on those of us within the scientific community to ensure their continued existence.

The Sandhills flora where the MHJB is found is both heavily defended from predation and herbivory and contains a wealth of species that are native to the Sandhills. Over 90 species of plants found here have been identified as "Sandhills specialty plants" and occur in one of the following categories; endemic, disjunct population, coastal relict, threatened, locally unique, or undescribed ecotypes (McGraw, 2004). Not only are many plants unique to the

Sandhills community, but variation has also been found to occur within the Sandhills flora species distribution as a result of spatial variation and the variety of interacting biotic and abiotic components (McGraw, 2004). The MHJB is found only among these highly specialized plants and nowhere else. Given the unique morphological characteristics of the MHJB and the uniqueness of the plant species found in the Sandhills, it is highly possible that the MHJB is a product of coevolution with one or several species of flora found within the Sandhills. Monitoring surveys suggest and current conservation management have used Ponderosa pine as an indicator of the MHJBs' habitat. It has also been suggested that MHJB host plant selection is limited to Ponderosa pine or plants in areas within a few hundred feet of Ponderosa pine trees (Arnold, 2004).

RESEARCH OBJECTIVES

The Recovery Plan for the endangered MHJB recommends that research focus on the MHJB's habitat requirements for long-term survival (e.g., feeding behavior requirements; requirements for larval and adult stages) (U.S. Fish and Wildlife Service, 1998). Although much is unknown about the MHJB, a crucial part of providing it full protection is to understand and protect female habitat and larval host plant(s). The MHJB is known to eat only in its fossorial, larval stage. The objectives of this research are to identify 1) vegetation associations with female burrows and male flight regions (see habitat association study) and 2) any host plants the larvae eat (see feeding study).

HYPOTHESES

In order to understand the MHJB's host plant selection, hypotheses were tested for each of the two foci of the study.

Objective I: Habitat Associations

Hypothesis 1: *Polyphylla barbata* coevolved with endemic sandhill plants and has specialized habitat needs within the greater sandhills plant community.

H_1 Plant microsite assemblages associated with MHJB (burrows or male MHJB flight regions) will represent a subset of the larger plant assemblage.

$H_{1.1}$ Plant species richness will be lower where MHJB is active (burrows or male MHJB flight regions) than in plant microsites where MHJB is not active.

$H_{1.2}$ Native Zayante sandhills plant species will be more abundant within microsites where MHJB is active(burrows or male MHJB flight regions) than in adjacent microsites where MHJB is not detected.

Hypothesis 2: Male and female MHJB select generally corresponding habitat.

H_2 Plant species richness and Native Zayante sandhills plant species abundance found in female MHJB burrow microsite plant assemblages will not differ detectably from those in male MHJB flight microsites.

Hypothesis 3: Female burrows will be located within 50 meters of the nearest mature *Pinus ponderosa.*

Objective II: Feeding

Hypothesis 4: The MHJB has evolved as a specialist feeder, selectively choosing host plants from within one family, genus or species of plant.

H_4: Frass from MHJB larvae will contain plant species from a limited range of plant taxa.

MATERIALS AND METHODS

Habitat Association

In order to achieve the first objective, I employed the widely accepted, standard technique of plot counts (Sutherland, 1996), comparing host plant microsites between known (female burrow and male flight regions) and unknown (MHJBs not observed) sites. This method is frequently used to analyze plant cover and was used to measure the vegetation type and density around the burrows prior to larval excavation.

Feeding

To achieve the second objective, a more novel approach of frass analysis was employed to identify host plant(s). As this procedure has not been validated with beetle larvae, the procedure will be considered exploratory in nature. Proportions of each plant type in the fecal pellets were recorded and summed for each plant species. Sample plants were gathered from the field site, pulverized, and used as reference material. To confirm that the MHJB larval frass was analyzed, the next step of identifying species involved DNA analysis. Insect DNA analysis is a relatively new technique for identifying insects. Insect nuclear ribosomal DNA has been used in insect evolutionary analysis as it is known to be a rapidly evolving gene region and is applicable to a wide range of invertebrate taxa (Slaney and Blair, 2001).

The methods chosen for this research have been used in several studies: frass analysis by Sword and Dopman (1999) and Dopman et al. (2002); vegetation analysis by Sutherland (1996), and insect DNA analysis by Slaney and Blair (2002). Sword and Dopman (1999) and Scholtens & Holland (1997) successfully determined grasshopper host plants by microscopically examining fecal (frass) pellets.

Research Site

Quail Hollow Quarry is 220 acres of disturbed land that supports eight plant communities, including northern maritime chaparral, successional scrub, central coast scrub, maritime coast range ponderosa pine forest, hardwood-conifer woodland, sand parkland, central coast live oak riparian forest, and central arroyo willow riparian forest (Granite Rock Company, 1998). This study site was chosen because of the "take" permit issued by the U.S. Fish and Wildlife Service for the property under the *Revised Habitat Conservation Plan* for Granite Rock Corporation, owners of the quarry. The quarry provides typical habitat for the MHJB. Many of plant communities are intergraded, as well as degraded, especially in the Future Mining Area of QHQ (O'Malley, et al. 2003). The

Quarry is located approximately 35 miles south and west of San Jose near Felton, CA. just south of East Zayante Road. The FMA of the quarry is a hill of variably disturbed land within the quarry with some portions being quite disturbed and other portions found are more or less intact.

Study Design and Procedures

Four procedures were followed to test the hypotheses stated above. The two procedures described below were used to identify MHJB habitat associations. The following two procedures were used in the larval feeding study.

Objective I. Habitat Association:

1. Identify potential larval burrows,
2. Identify the vegetation surrounding female burrows, male MHJB flight regions, and sites where MHJB were not observed. Measure the distance from female burrow sites to the nearest *Pinus ponderosa.*

Procedure 1: Identify potential larval burrows

The male MHJB has a very limited flight season and is most active between 20:30 and 21:45 between mid-May through mid-August with some year to year variation. MHJBs were observed three nights per week from the first night of observed male flight in June to the last night of flight observations in July. The purpose of this activity was to identify the range of female burrow locations, describe the vegetation surrounding the female burrows, and mark the female burrows for later excavation of larvae. All flight activity was monitored with flashlights. Flashlights were used as infrequently as possible in order not to disturb the flight patterns of the males. The terrain within the FMA is steep, hilly, and often covered with dense brush and stands of poison oak, making some areas inaccessible for nighttime observations. Due to the nature of the terrain within the FMA, male flight activity and female burrow locations were located haphazardly.

MHJB matings were observed during 7 nights in 2004 and 10 nights in 2005. A Global Positioning System was used to obtain UTM coordinates, also conditions in the quarry at the time of the observations were recorded for each night of observations (See field notes, Appendix C). During nights of mating observations, temperatures ranged between 12 and 21 °C. Wind speeds ranged from 0 to 13 kph. Male beetles were observed to fly and mate under clear and cloudy skies (0 to 90% cloud cover).

Procedure 2: Identify the vegetation surrounding female burrows, male MHJB flight regions, and sites where MHJB were not observed. Measure distance from female burrows to nearest Pinus ponderosa.

To assess the habitat needs for the MHJB larvae, microhabitat plant availability was measured by estimating the cover and abundance of perennial plants available to the beetle larvae year round within each of the eighteen $1m^2$ potential female burrow sites. The proportion of each plant species found within the $1m^2$ quadrats was estimated by placing a frame over the area. The frame was divided into a 10 x 10 grid to facilitate percentage estimations. Plant cover and abundance were recorded for 44 stratified-randomly placed $1m^2$ quadrats. Eighteen of these quadrats were placed in areas where the MHJB were known to occur (in areas adjacent to where burrow sites were found, up to 80m away from burrow plots). These areas are important to understanding of the MHJB habitat as they are regions where matings between male and female MHJBs may have occurred but were not observed, although males were seen flying in the vegetation and over these areas during nights that matings were observed. Twenty-six quadrats were stratified-randomly placed in areas of the FMA where it was not known if the beetles occurred (see Figure 3 for known and unknown areas). These regions were adjacent to areas where MHJBs were frequently observed. However, these areas were also searched for MHJBs with flashlights in the same manner as other regions where MHJBs were observed but none were observed in these areas, thus they were labeled as areas of unknown occurrence. Also, to further understand the potential requirement and presence of *Pinus ponderosa* in the MHJB habitat, the distance between each female burrow site and the nearest mature Pinus ponderosa was measured.

Objective II. Feeding Analysis Procedures:

3. Collection of larval tissue and frass samples from larvae found in the excavated burrows identified in step #1,
4. Analyze larval DNA to verify species.

Procedure 3: Collection of larval tissue and frass samples from larva collected from the potential larval burrows

Previously tagged female MHJB burrows were excavated in late spring (June 2005) and late summer (September 2005) of the year following summer observations. MHJB larvae were likely to be at least 2 cm long at this point (i.e., in their 3rd or 4th instar) and were easily identified within the soil. Burrows areas were excavated to 1m deep. Frass was collected from captured larvae by placing the beetle larvae in a screen covered vial (to allow for air circulation) in a warm dark place for up to 6 hours or until individuals deposited at least 1 frass pellet. Collected frass pellets were stored frozen for later microscope analysis. Because the larvae are morphologically indistinguishable from co-occuring *Polyphylla* larvae, DNA analysis was used to distinguish the species from one another. Larvae from which a frass sample was collected were preserved in 95% ethanol for later DNA analysis

Procedure 4: Analysis of larval DNA

For molecular identification of the larvae, one adult and nine larval (including those larvae that had produced frass) MHJB larvae were captured from within the FMA. For genetic comparison, two *Polyphylla decemelineata* adult tissue samples from the FMA of QHQ and from Riverside County's San Jacinto Mountains were collected. Partial fragments of the ITS2 (internal transcribed spacer region 2 of the nuclear ribosomal DNA) were sequenced to differentiate the species. The ITS2 gene is a rapidly evolving gene and lends itself to identification of finer gradations of speciation within a genus (Slaney & Blair, 2000). Preserved tissue samples were macerated and incubated with proteinase-K and 5% Chelex solution at 55°C for one hour followed by incubation at 100°C for 8 minutes. Primers used to generate partial fragments of the nuclear ITS2 region were ITS2-55 and R2 (5′ TGT GAA CTG CAG GAC ACA TG 3′ and 5′ TCT CGC CTG CTC TGA GGT 3′, respectively). Fragments were amplified by PCR (polymerase chain reaction) in a total volume of 21μl (11.2 μl deionized water, 2.0 μl 10 x PCR buffer, 0.4 μl 10mM dNTP mixture, 0.5 μl of each primer, 4 μl Q (betane) solution, 2 μl template DNA, and 0.5 μl Taq DNA polymerase). PCR amplification was performed in a CycleLR Genomyx thermal cycler. The mixture was incubated at 95°C for 3 minutes for initial denaturation, followed by 30 cycles of 94°C for 45 seconds, primers annealing for 45 seconds at 55°C, and extension at 72°C for 1 minute. This was followed by an end run of 7 minutes at 72°C to complete elongation. Amplification products were visualized by electrophoresis in a 1% agarose gel in a 1 x TAE bromide (0.5 μg m^{-1}). Purified PCR products were sequenced on San Diego State University's ABI Prism® 3100 Genetic Analyzer (Applied Biosystems) using Big Dye Terminator® Chemistry, version 3.1. All sequences were compared for positive species identification of the MHJB.

DATA ANALYSES

Objective I. Habitat Association Analysis

Several analytic procedures were employed to analyze burrow site vegetation. Initially, descriptive statistics were recorded for the three sample terrains (burrow, male MHJB flight region sites, and sites where MHJB were not observed). For further analyses, terrain type was treated as the independent variable and the proportion of plant species per square meter for each site type were the dependent variables. As a combined variable, female burrow sites and male flight regions, was compared to areas where MHJB was not detected. Analysis of variance test (ANOVA) was completed in order to compare individual plant species for each of the three site types.

An independent samples t-test analysis was conducted in order to compare the abundance of Zayante Sandhills plant present in microsites where MHJB is active to sites where MHJB was not detected. These species included: *Arctostaphylos tomentosa ssp.crinita, Baccharis pilularis, Ceanothus cuneatus, Chorizanthe pungens var. hartwegiana, Ericameria ericoides, Eriogonum nudum, Lotus scoparius, Lupinus albifrons, Mimulus aurantiacus, Pinus ponderosa, Pteridium aquilinum, Salvia mellifera* and as a group all Other (*Eschoscholzia californica, Gnaphalium sp., Hamata sp., Heterotheca grandifolia, Holocarpha sp., Lessingia* *filaginifolia var. filaginifolia*) species were included. Female MHJB burrow sites were compared to male MHJB flight regions for the grouped variable of native Zayante Sandhills plant species as described above. For ANOVA and t-test analyses, non-normally distributed variables were transformed into normally distributed (Z-score) continuous variables to meet the data requirements of these procedures. All statistical analyses were completed using SPSS statistical software ver. 11. (SPSS, 1999).

Objective II. Feeding Study Analysis

Eight female burrows found during the summer of 2004 were excavated in June 2005 and one burrow found in July 2005 was excavated in September 2005. Five burrow locations yielded larvae (Indicated by blue arrows in Figure 2). In total, twelve larvae were excavated from the FMA. Of the larvae excavated, six produced frass that was stored in sterile containers and frozen for later identification. Following frass production procedures, larvae were placed in containers of soil. Seven larvae died soon after excavation and were place in 95% ethanol. Two of the seven larvae that died and did not produce frass were preserved for morphological identification. Five larvae were later placed in 95%

ethanol in preparation for DNA analysis. Details of the vegetation of burrows that yielded larvae are provided in Appendix A.

In total, ten larval and three comparison adult beetles captured from the field site underwent DNA analysis. The three adult beetles were two *Polyphylla decemlineata* and one *Polyphylla barbata.* Five of the ten larvae's body tissue degraded after being placed in ethanol, reducing the potential overall yield from the DNA analysis. DNA was successfully extracted from five of the ten larvae by using portions of maxillary palps and tarsal segments.

Larval frass was qualitatively compared to ground plant roots collected from the FMA of QHQ for positive identification of host selection. Six frass samples were collected in June and September 2005 from larval burrows within the FMA. Analysis of plant fragments in fecal samples included comparison of the epidermis and cell wall structure of the reference plant specimen to the frass pellets teased apart on a microscope slide. This method of analysis has been successfully employed in the identification of insect host plant selection. For each plant root type and frass pellet a photograph was taken (Appendix D and E). The author first prepared slides of frass pellets and freshly collected plant root tissue that had been macerated in preparation for microscopic examination. For easier comparisons between frass and potential plant, micrographs were taken of portions of each prepared slide at 100x magnification. The frass pellets were analyzed by plant anatomist David Bruck for remains of plant tissue and other materials. The plant composition in the frass was compared by the author to plant species available to the larvae in the vicinity from which they were captured. To determine the presence of mycorrhizae, a chitin specific stain, lactophenol cotton blue, was applied to the frass material.

RESULTS

Objective I. Habitat Association

The results for the habitat association are divided into two sections. First, the total number of observations made of the MHJB is described and the vegetation for the three plot site types is compared and contrasted. Next, the vegetation analyses results of for hypothesis one are described.

Identification of potential larval burrows. Observations were conducted during the summer months (June, July, and August) of 2004 and 2005 for a total of 44 observation periods (21 in 2004, 23 in 2005). A total of 19 MHJB matings and female burrows were located within the FMA of QHQ (9 in 2004, 10 in 2005) (See Appendix C for field notes). The aerial photograph in Figure 2 indicates areas where burrow were found (outlined in yellow) within the FMA. The areas in which MHJBs were observed to occur and not occur are highlighted in Figure 3.

Descriptive analyses for vegetation sample sites. For all sample variables, non-transformed scores will be reported to facilitate easier interpretation of findings. In total, 16 plant species were found within burrow sites. Nineteen plant species were found in the male MHJB flight regions and 23 plant species were found in areas where MHJBs were not observed. See Table 1 for means and standard deviations of each plant species found in female burrow, MHJB flight regions, and sites where MHJB were not observed. For raw scores for each plot, see Appendices A and B.

Table 1: Analysis of Variance for Plant Species Coverage per Site Type

	Burrow (n =18)		MHJB Flight Regions (n=18)		MHJB Not observed (n =26)	
Plant Species	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Arbutus menziesii	0.00	0.00	0.00	0.00	2.15	10.20
Arctostaphlyos tomentosa	0.00	0.00	0.00	0.00	6.04	21.32
Baccharus pilularis	0.00	0.00	0.60	2.53	8.38	25.02
Bare ground[a]	49.33	30.96	16.49	29.40	25.23	33.94
Ceanothus cuneatus	1.44	6.13	9.18	26.27	3.85	19.61
Chorizanthe pungens var. hartwegiana[b]	4.44	10.21	0.26	1.12	0.03	0.13
Ericameria ericoides	1.77	5.82	7.22	19.16	0.50	2.55
Eriodictum californica	0.44	1.34	0.11	0.47	0.73	3.34
Eriogonum nudum	4.83	20.01	0.07	0.21	0.04	0.20
Lotus scoparius	5.24	7.53	9.42	20.74	6.38	16.20
Lupinus chamissonis[c]	0.17	0.71	0.17	0.71	4.67	13.33
Mimulus aurantiacus	6.36	14.97	3.67	7.71	1.88	5.11
Moss	1.28	3.68	0.53	2.24	1.73	8.83
Other*	2.30	4.05	0.50	0.79	4.42	16.00
Pinus ponderosa[d]	0.00	0.00	0.00	0.00	9.42	27.86
Poaceae	20.76	36.95	34.11	42.81	21.58	34.61
Pteridium aquilinum	1.06	3.30	5.81	21.39	0.23	1.18
Salvia mellifera	0.58	2.48	5.56	23.57	1.77	9.02

* includes *Eschoscholzia californica, Gnaphalium sp., Hamata sp., Heterotheca grandifolia, Holocarpha sp., Lessingia filaginifolia var. filaginifolia* and dead wood

[a] Significant differences between burrow sites, flight regions, and sites where MHJB were not observed (p= .009)

[b] Significant differences between burrow sites and sites where MHJBs were not observed (p = .034)

[c] Significant difference between the combined variables of burrow and flight regions, and sites where MHJBs were not observed (p = .047)

[d] Significant differences between the combined variables of burrow and flight regions, and sites where MHJBs were not observed (p = .046)

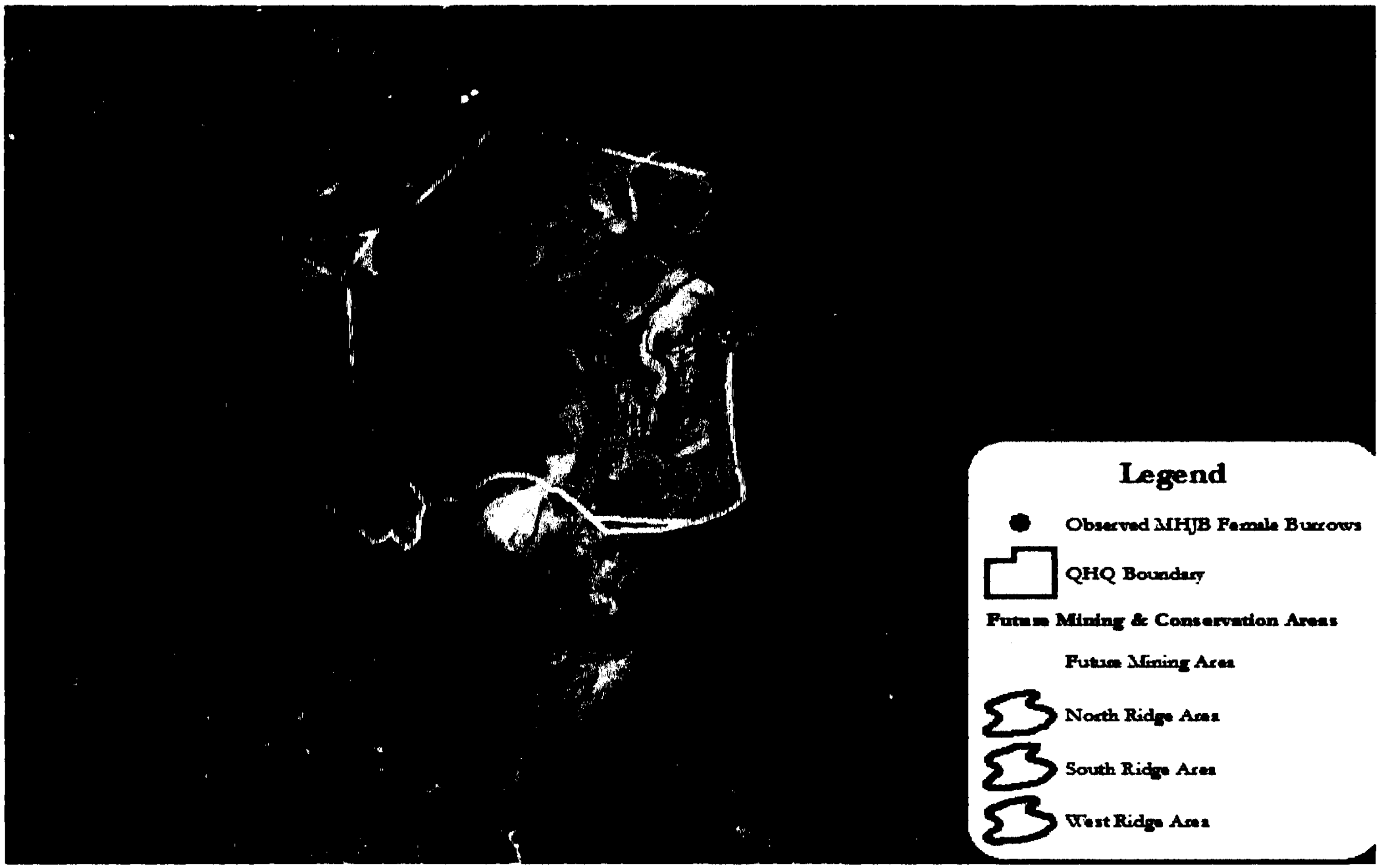


Figure 2 : Location of female MHJB burrows within the FMA

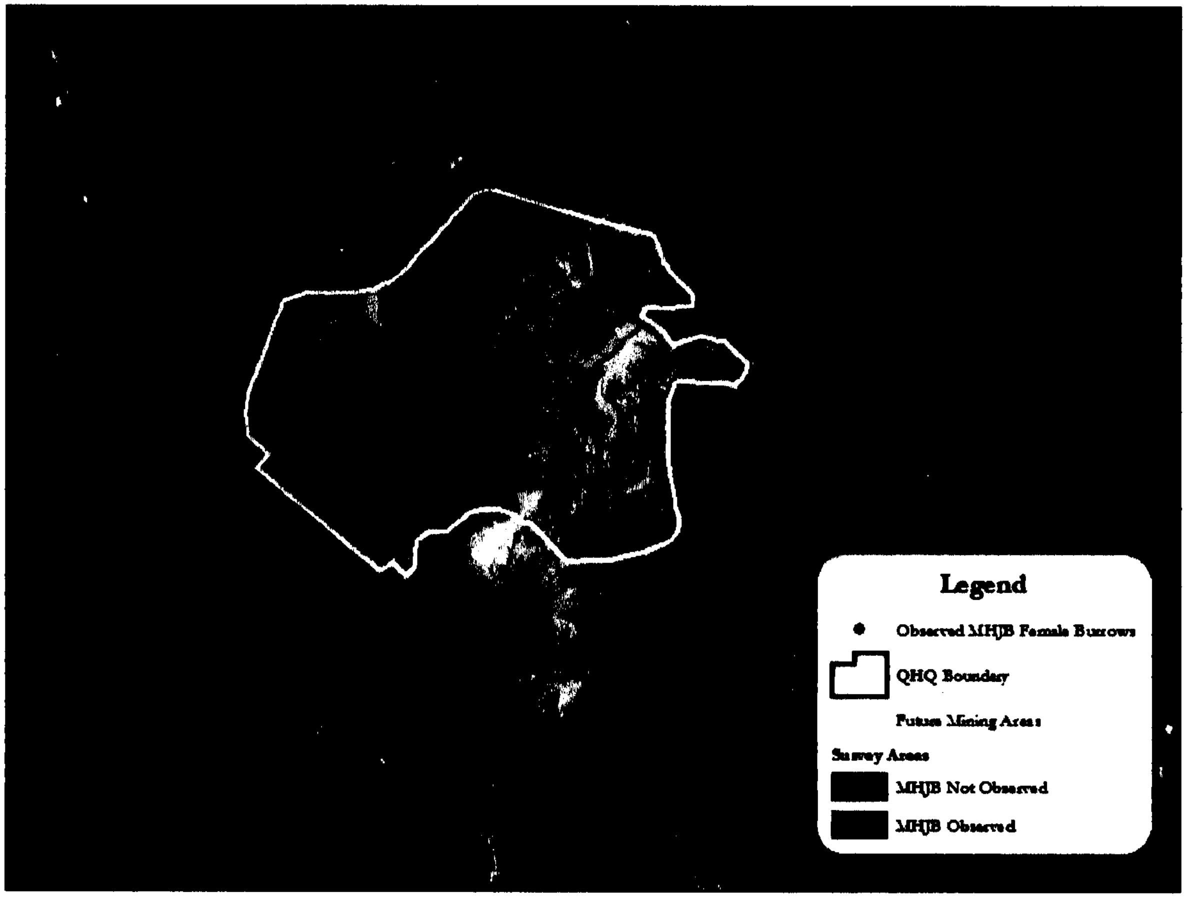


Figure 3 : Surveyed areas within the FMA

On average, the greatest proportion of each of the eighteen $1m^2$ burrow sites was covered by 49% bare ground. In contrast, each male MHJB flight site sampled averaged 16% bare ground, and each site where MHJBs were not observed were averaged 25% bare ground. The vegetation surrounding the burrows was found to be mostly grass species (*Poaceae sp.*) which, on average, covered 21% of each plot, in contrast, grasses were found more frequently in male MHJB flight regions covering, on average 34% of each plot. Similar to burrow plots, grasses were found to cover 22% of each plot where MHJBs were not observed on average.

A number of perennial shrubs and herbs, and trees were found to have variable portions of each of all site types. *Eriogonum nudum,* and *Chorizanthe pungens var. hartwegiana* each averaged between 4 and 5% cover of each burrow plot but less than 1% for male MHJB flight regions and areas where MHJBs were not observed. *Ceanothus cuneatus* averaged 2% of each burrow site, 9% of male MHJB flight region sites, and 4% in sites where MHJBs were not observed. *Lotus scoparius* averaged 5% of each burrow plot, 9% of male MHJB flight regions, and 6% of each site where MHJBs were not observed. *Eriodictyon californicum, Lupinus chamissonis,* moss, *Saliva mellifera, Pteridium aquilinum* each averaged less than 2% of each burrow site. *Lupinus chamissonis* averaged less than 1% of each male MHJB flight region site and 5% of each unknown site. *Saliva mellifera* was more abundant in male MHJB flight region sites (6% on average) and less abundant in areas where MHJBs were not observed (2%). *Pteridium aquilinum* and *Quercus agrifolia* each averaged 6% of male MHJB flight region sites, *Quercus sp.* were not found in burrow sites or sites where MHJBs were not observed. *Ericameria ericoides* covered, on average, 7% of each male MHJB flight region site and on average; it was found to cover 2% of each burrow site and 1% of each unknown site. *Mimulus aurantiacus* was found on average to cover 6% of burrow sites, 4% of male MHJB flight region sites, and 2% of areas where MHJBs were not observed. *Baccharus pilularus* was not found in burrow sites, but was found in MHJB flight regions (averaging less than 1%) and more frequently in areas where MHJBs were not observed (8%).*Eriodictum californica* averages less than 1% in all plots. *Moss sp* averaged less than 2% all sites.

Other annual plant species or features that otherwise would go undescribed were grouped into one category (including *Eschoscholzia californica, Gnaphalium sp., Hamata sp., Heterotheca grandifolia, Holocarpha sp., Lessingia filaginifolia* and dead wood) had a combined average of 4% on sites where MHJBs were not observed, less than 3% of cover within each burrow site, and less than 1% in male MHJB flight regions.

Several species were found in the unknown plots that were not found in the burrow sites or male MHJB flight regions, these included *Pinus ponderosa* which covered an average of 9% of site where MHJBs were not observed, followed by *Arctostaphlyos tomentosa* 6% and *Arbutus menziesii* (2%). *Salix spp.* averaged 2% in sites where MHJB were not observed.

For the majority (11) of female burrow locations, *Pinus ponderosa* was found within 26 meters (85ft). Four female burrow locations were found between 51 and 59 meters (173 -193 ft) away from the nearest mature *Pinus ponderosa*. Two burrow locations were found between 134 and 137 meters (441-449 ft) from the nearest *Pinus ponderosa* (see Table 2). The mean distance between female burrows and the nearest *Pinus ponderosa* was 35.92 meters (S.D. 41.86).

Table 2: Female MHJB Burrow Sites, Distance to Nearest Mature *Pinus ponderosa*

Burrow	Location	Distance (m) to *Pinus ponderosa*
6	1st Hill	5.79
9	Backhill	7.32
8	Backhill	9.45
10	Farside	10.36
4	1st Hill	10.97
5	1st Hill	11.89
11	Farside	13.72
3	1st Hill	14.33
2	1st Hill	15.24
1	Scrub Oak Hill	15.85
7	Backhill	16.76
12	Farside	25.60
13	Farside	52.73
16	Drypond	58.83
15	Drypond	59.44
14	Drypond	62.79
17	Wetpond	134.42
18	Wetpond	136.86

$H_{1.1}$ Plant species richness will be lower where MHJB is active (burrows or male MHJB flight regions) than in plant microsites where MHJB is not active.

As a combined variable (female MHJB burrow sites and male MHJB flight regions) ANOVA analyses detected differences from areas where MHJB was not observed in the abundance of *Lupinus albifrons* ($F = 4.128$, $df = 1$, $p = 0.047$) and *Pinus ponderosa* ($F = 4.145$, $df = 1$, $p = 0.046$) (See Figure 4). Both species were more likely to be present in areas where MHJBs were not detected (Table 1). In total, fewer species were found in areas where MHJBs were detected supporting hypothesis 1.1 and indicating that the MHJB selects habitat that includes a subset of plant species within the Sandhills environment.

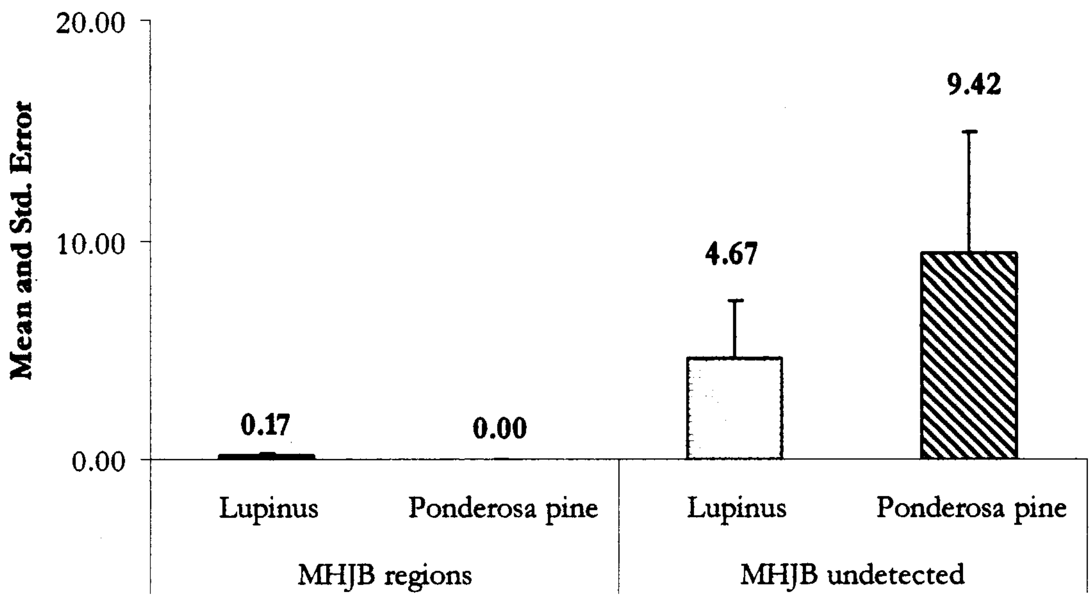


Figure 4 : Mean proportion of *Lupinus albifrons* (p = .047) and *Pinus ponderosa* (p = .046) in regions where MHJBs were observed and undetected.

Related to the first hypothesis, a three way analyses (ANOVA) was conducted between the three site types. Significant differences were found between groups for *Chorizanthe pungens var. hartwegiana* ($F = 3.937$, $df = 2$, $p = 0.025$). To reduce the risk of Type 1 errors, the p values were adjusted using *post hoc* Bonferroni procedure. Bonferroni corrections revealed significant differences between the proportions of *Chorizanthe pungens var. hartwegiana* found in female burrow sites and sites where MHJBs were not observed ($p = 0.034$) but not between female burrow sites and MHJB flight regions or MHJB flight regions and areas where MHJBs were not observed (Figure 5).

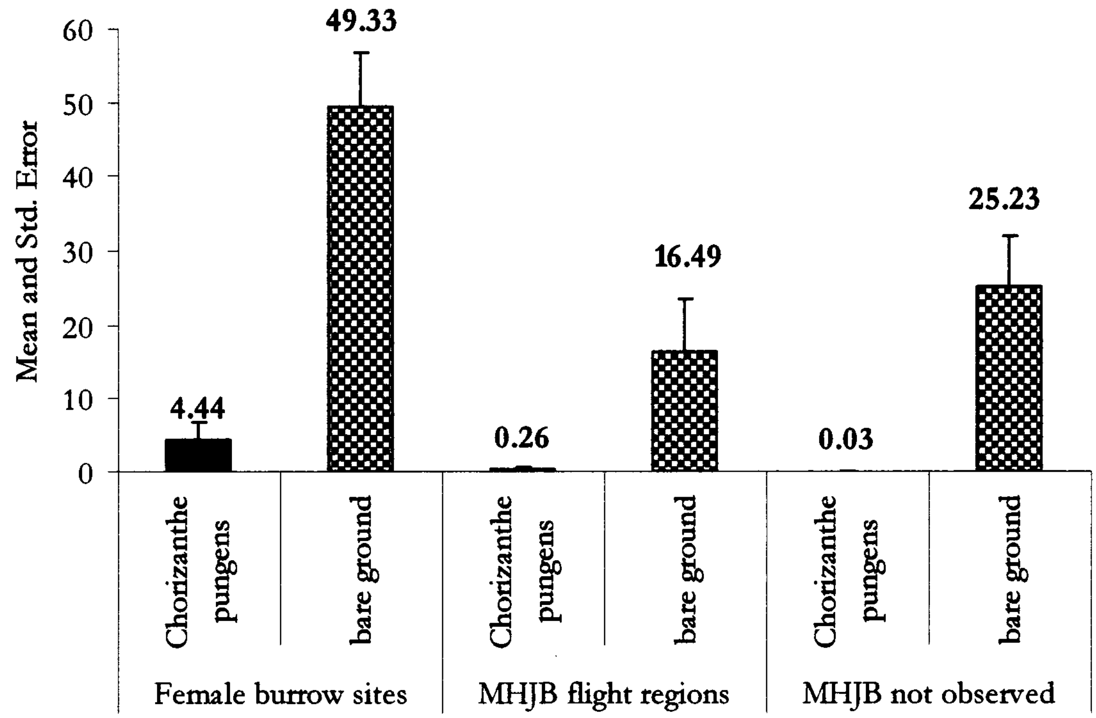


Figure 5 : Mean proportion of *Chorizanthe pungens var. hartwegiana* (p=.025) and bare ground (p = .008), per plot type.

Significant differences were also found between groups for the amount of bare ground ($F = 5.229$, $df = 2$, $p = 0.008$) (Figure 5). Bonferroni corrections of bare ground indicated significant differences between female burrow sites and MHJB flight regions ($p = 0.009$) and between female burrow sites and MHJB flight regions ($p = 0.049$). Bare ground occurred with greater frequency within burrow sites as opposed to male MHJB flight regions ($p=0.049$) and also with greater frequency in burrow sites as compared to sites where MHJB were not observed ($p=0.008$). The mean percent of each plant species or feature (i.e., bare ground or other features) found within burrow, male MHJB flight regions, and sites where MHJB were not observed are represented in Table 1.

$H_{1.2}$ Native Zayante sandhills plant species will be more abundant within microsites where MHJB is active than in adjacent microsites where MHJB is not detected.

No significant difference was detected between the abundance (area per square meter) of native Zayante Sandhills species where MHJBs were present (female MHJB burrows and male MHJB flight regions) or absent ($t = -8.19$, $df = 60$, $p= 0.10$). The majority of species found within the study area (excluding *Arbutus menziesii* which is not listed as native to the Zayante Sandhills although it is native to California (McGraw, 2004), and *Poaceae sp.*) were native to the Sandhills environment (Table 1).

H_2 Plant species richness and Native Zayante sandhills plant species abundance found in female MHJB burrow microsite plant assemblages will not differ detectably from those in male MHJB flight microsites.

No significant differences were found between the two groups ($t = -.227$, $df = 34$, $p = 0.822$) (Table 1).

H_3 Female burrows will be located within 50 meters of the nearest Pinus ponderosa

The majority of female burrows (11 of 18) were found within 26 meters of nearest *Pinus ponderosa.* Six female burrows found between approximately 50 and 59 meters from nearest *Pinus ponderosa.* Two female burrows were found between approximately 134 and 137 meters from nearest *Pinus ponderosa.* The mean distance from female burrow to the nearest Pinus ponderosa was 36 meters (Std.Dev. = 42 meters) (Figure 6).

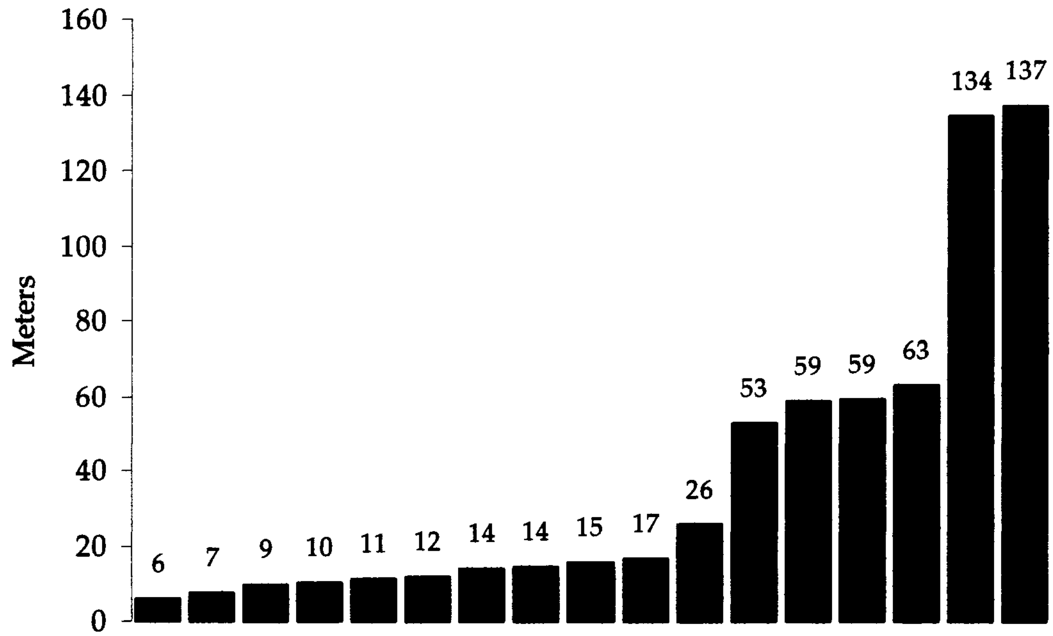


Figure 6 : Distance in meters from nearest Pinus ponderosa to female MHJB burrow locations

Objective II. Feeding Study

The results for the feeding study are divided into two sections. The first section describes the total number of larval beetles found at the burrow sites and the resultant DNA analyses. Second, the frass analyses results for hypothesis two are described with their DNA comparison.

Collection of larval tissue and frass. All five larvae that were successfully sampled were identified as *Polyphylla barbata* with no intra-population variation for the ITS2 gene sequence. The *Polyphylla barbata* populations differed from the *Polyphylla decemlineata* populations sampled by eight base pairs and three base insertions. See Appendix F for DNA sequences.

Hypothesis 3: The MHJB has evolved as a specialist feeder, selectively choosing host plants from within one family, genus or species of plant.

Plant anatomist, David Bruck, was able to identify materials within six frass pellets. Bruck identified material indicative of primary growth, including vessel elements, pitted elements, and circular xylem in frass pellet from larva #S671. Also found within the frass of # S671, # S692, and pellet with unidentified DNA (#S669b) were helical elements (xylem vessel), and multicellular hairs. In pellet #S673 he identified fibers and multicellular hairs. In the same pellet mentioned above, with unidentified DNA (S669b), he identified border pits and oval apertures, many vessel elements, branched hairs (indicative of stem structures) and materials that may have been indicative of rhizomes. This pellet was of interest because it was collected from a larva that was excavated from the same location as a positively identified *Polyphylla barbata* larva (S669a) (Table 3). Much of these materials (4 of 5 pellets) were indicative of primary non-woody growth. All of these materials are indicative of the stems of flowering angiosperms.

In pellet #S692 *Pteridium aquilinum* was possibly identified, as it contained anatomical features that when found together in one plant make it unique to bracken fern (both scalariform pitting and xylem vessel elements). This pellet also contained trichomes which were possibly indicative of the underground stem portion of the plant.

Many materials found within the frass pellets did not polarize when exposed to a microscope polarizing lens (fragments found within 4 of 5 pellets), suggesting that they did not have mechanisms of plant cells that make use of photosynthesis. This material was identified as fungal hyphae with the chitin indicator stain. In general, all pellets indicated that the larvae were eating primary non-woody growth indicating root shoots or young stems (Table 3).

Table 3: Frass Analysis, Plant and Mycorrhizal Structures Identified

Larval DNA Identifier	**Structural elements identified**	**Conclusion**
S669a	Fungal structures	Mycorrhizae
S669b No DNA**	Border pits, oval apertures, helical elements, multicellular branched hair	Angiosperm
S671	Non-woody herbaceous material. No periderm, no wood present. Circular xylem present. Primary growth indicated by vessel elements present. Pitted elements and multicellular hairs.	Angiosperm
S672	Possible root cells, wood and xylem, fungal structures	Angiosperm, mycorrhizae
S673	Fibers, multicellular hairs, fungal structures	Angiosperm stem structure, mycorrhizae
S692	Trichomes, xylem, helical elements, multicellular hairs, scalariform pitting, vessel elements, pitted elements	*Pteridium aquilinum*

** This larva was collected from the same burrow site as S669. (See Appendix 3 for possible plant species)

DISCUSSION

The primary aim of this investigation was to identify host plant selection of the MHJB. Two approaches were employed to identify host plants. The first approach, using vegetation analysis, provided mixed results. The hypothesis 1.1 predicted that plant microsite assemblages associated with the MHJB (female burrows or male MHJB flight regions) will represent a subset of the larger plant assemblage was supported. Two species of plants (*Lupinus albifrons* and *Pinus ponderosa*) occurred with greater frequency outside of areas where MHJBs were detected. However, native Zayante Sandhills plant species were not found to be more abundant within microsites where the MHJB is active when compared to microsites where the MHJB was not detected.

ANOVA and post hoc comparisons adjusted with a Bonferroni correction revealed that the amount of *Chorizanthe pungens var. hartwegiana* differed between burrows and sites where MHJB were not observed and that the amount of bare ground differed between all three sites. *Chorizanthe pungens var. hartwegiana* was not found consistently within all burrow plots (It was found in four burrow sites in percentages of 6%, 13%, 25%, 36%.), and bare ground was found in fourteen sites in proportions ranging from 25 to 83 percent. Aside from these differences, no clear pattern was found between the sites.

Furthermore no differences were found between the percent coverage of native Zayante Sandhills plant species in MHJB burrows and male MHJB flight regions which was predicted by hypothesis two. Overall, the MHJB is found within a variety of vegetation within the FMA; plant species types and abundances varied among burrow sites.

Frass analysis results proved more definitive with respect to host plant selection. Hypothesis four was not confirmed results from this study, and all indications are that MHJB is not a specialist. Rather, materials found within the frass reveal distinctly different species of plants being consumed by the larvae and mycorrhizae, a fungus and a viable source of protein. Possibly identified within the frass, due to its unique anatomical composition, was *Pteridium aquilinum*. *Pteridium aquilinum* was found in only two of the burrow sites and it was found in the frass of a larva found proximal to a plant, suggesting that the MHJB is making use of what is widely available. Interestingly, *Pteridium aquilinum* is known to contain secondary compounds and chemicals that may interfere with insect growth (Jones and Firn, 1978). Regardless, *Pteridium aqulinum* is host to many insect species worldwide.

Other materials within the frass were identified as flowering angiosperms. On the basis of what was available in burrow plots, host species identified within the frass could include *Lotus scoparus, Mimulus aurantiacus, Hetereotheca grandiflora* and *Ericameria ericoides.* Although the results of the analysis of the frass samples are interesting, only six frass samples were available for evaluation and evaluation was difficult. It is possible that the analysis of additional samples would reveal a wider diet. It is also possible that some host species did not yield a positive identification as the material may been altered by consumption beyond distinction. Clearer procedures need to be developed for larval beetle frass analyses. The small amount of larvae collected may be the result of a limited population or limits of the excavation process.

Polyphylla research indicates that most species within the genus feed on the roots of many plants, and larvae are found in association with sandy soils (Young, 1988). Several other *Polyphylla* species are known to be economic pests, feeding on the roots of fruit trees, and as adults feeding on the leaves of forest trees such as elm, maple and oak. *Polyphylla decemelineata,* the MHJBs sympatric congener, and an economic pest in parts of central and southern California, is also said to feed on the needles of ponderosa pine trees as an adult (Evans and

Hogue, 2004). Given that many *Polyphylla* are evidenced to be generalists, it is feasible that the MHJB feeds on several host plants within the Sandhills.

Why the MHJB is found in some sites but not others cannot be answered by the results of this study. The number of plots sampled was relatively small, the host plant feeding preference possibly were not resolvable if such differences exist. Given the number of plots sampled, the study had 80% power to detect an effect size of 0.73. It is unknown what effect size is meaningful.

MHJB dispersion was not limited to undisturbed soils. Burrows were found in areas where native disturbance adapted species such as *Heterotheca grandiflora* proliferated. Vegetation in areas where MHJB matings were observed consisted of degraded and disturbed parkland, silver-leaf manzanita mixed chaparral with *Pinus ponderosa,* and a mélange of degraded parkland (Jodi McGraw, pers.comm 7/12/2005). All but four burrow sites that were found in the relatively intact area of the far side of the FMA were found in degraded Sandhills habitat. There also could be other chemical or ecological factors and relationships with predators or parasites that may be determining the MHJBs bionomics. In conservation management, *Pinus ponderosa* has been used as an indicator of MHJB habitat. It has also been suggested in unpublished reports, that it is a potential host plant of the MHJB (Arnold, 2004). Although *Pinus* *ponderosa* was not found to be a host plant in this study, it may still be a host plant for the species. Although many burrows were found near *Pinus ponderosa,* some observations and female burrows were also found in locations over 122 meters away from the nearest mature *Pinus ponderosa.* The habitat within the FMA is disturbed and not representative of an intact environment in which MHJBs would typically be found. We can be certain that within the Sandhills ecosystem, the MHJB can frequently be found proximally to *Pinus ponderosa.* However, in light of the current findings, *Pinus ponderosa* may be indicative of a level of ecosystem integrity (i.e., habitat with mature trees) where MHJBs may be found, but the MHJB is not limited to habitat proximal to *Pinus ponderosa.*

This study was strengthened by the use of multiple approaches. It effectively demonstrated the efficiency of using field observations and collections of larvae from potential female burrows by yielding only MHJB larvae from female burrow sites. The frass analyses were successful in identifying plants from two distinctly different classes and also fungi, an entirely separate kingdom. The study was limited by the small sample sizes (both vegetation and frass) and infrequent field observations. Further, the study is limited by the sites that were defined as areas where MHJB was not detected. The terrain within these areas may not have been distinguishable from areas in which the MHJB was detected. Areas where the MHJB was not detected may, in fact, be habitat for MHJB but were simply not detected by observation.

Future research

In order to better understand the MHJB, larger samples of vegetation analysis of burrow sites could refine and or confirm current findings. Also, analysis of frass could refine present findings and further define host plant selection. In terms of its recovery, it would helpful to understand how the MHJB colonizes an area, and key factors for its recolonization (i.e., proximity to intact/undisturbed area, characteristics of intact area). Further, the MHJB could benefit from studies of its relationship with other animal species that may affect its survival, such as potentially parasitic flies.

CONCLUSION

Understanding host plants is only one important part of protecting an endangered species. For the MHJB, a species that feeds only in its fossorial larval form, has a limited flight season and as yet is morphologically unidentifiable in larval form, combined genetic and frass analysis were useful methods for discerning host plant selection. The MHJB could benefit from continued studies of its life cycle, reproduction, and interaction with the surrounding complex and fragile Sandhills environment. Based on the findings from this study, I would recommend that *Pinus ponderosa* not be planted to maintain the MHJB habitat. Instead, I would recommend that potential habitat areas found further than 122 meters away from the nearest *Pinus ponderosa* need protection. Proximity to *Pinus ponderosa* is not necessarily a determinant of MHJB habitat. Proper protection of the MHJB would be benefited by continued monitoring and improvement of our knowledge of its life history. Maintenance of MHJB habitat by identification of typical microhabitat species associations should help the recovery of the MHJB. However, because the MHJB is not limited to one particular host plant species, it is important to protect the remaining Sandhills ecosystem to ensure population fitness. Habitat selection criteria could be refined with longer-term studies of vegetation analyses of female burrow sites and male MHJB flight regions. Further, public education and support for all those involved in the Sandhills recovery is crucial to the protection of this unique and fragile environment.

REFERENCES

Arnold, R.A. (1999). *1999 Monitoring report for the Mount Hermon June Beetle and Zayante Band Winged grasshopper at Hanson Aggregates' Felton Quarry.* Report prepared for Hanson Aggregates Mid-Pacific. Entomological Consulting Services, Ltd. Pleasant Hill, CA. 33 pgs.

Arnold, R.A. (2000). *Monitoring Report for the Mount Hermon June Beetle at the Quail Hollow Quarry in 2000.* Unpublished manuscript, Santa Cruz, CA. Report prepared for Hanson Aggregates Mid-Pacific. Entomological Consulting Services, Ltd. Pleasant Hill, CA. 15 pgs.

Arnold, R.A. (2004). *Mount Hermon June Beetle (Polyphylla barbata Cazier 1938).* In McGraw, J. M. (Ed.) *The Sandhills conservation and management plan: a strategy for preserving native biodiversity in the Santa Cruz Sandhills.*(Section 5.6 pp.92-99)Santa Cruz, CA: The Land Trust of Santa Cruz County.

Becerra, J. X. (2003). Synchronous coadaptation in an ancient case of herbivory. *Proceedings of the National Academy of Sciences , 100* (22): 12804-12807.

Bernays, E. A. (1991). Evolution of insect morphology in relation to plants. *Philosophical Transactions of the Royal Society of London B, 333,* 257-264.

Bernays, E. A. (1998a). Evolution of feeding behavior in insect herbivores, success seen as different ways to eat without being eaten. *BioScience, 48*(1), 35-44.

Bernays, E. A. (1998b). The value of being a resource specialist: behavioral support for a neural hypothesis. *The American Naturalist, 151*(5), 451-464.

Bernays, E. A. (2001). Neural limitations in phytophagous insects: implications for diet breadth and evolution of host affiliation. *Annual Reviews of Entomology, 46,* 703-727.

Borror, D. J., Delong, D. M., & Triplehorn, C. A. (1976). *An Introduction to the Study of Insects* (Fourth ed.). San Francisco: Holt, Rinehart, and Winston.

Connor, E. F., Hafernik, J., Levy, J., Moore, V. L., & Rickman, J. K. (2003). Insect conservation in an urban biodiversity hotspot: The San Francisco Bay Area. *Journal of Insect Conservation, 6,* 247-259.

Ehrlich, P. R., & Raven, P. H. (1964). Butterflies and plants: a study in coevolution. *Evolution, 18*(4), 586-608.

Futuyma, D. J. (1995). Genetic constraints on macroevolution: the evolution of host affiliation in the leaf beetle genus *Ophraella*. *Evolution, 49*(5), 797-809.

Futuyma, D. J. (2000). Some current approaches to the evolution of plant-herbivore interactions. *Plant Species Biology, 15*, 1-9.

Granite Rock Company. (1998). *Revised Habitat Conservation Plan*. Watsonville, CA: Granite Rock Company.

Griffin, J. R. (1964). Isolated *Pinus ponderosa* forests on sandy soils near Santa Cruz, California. *Ecology, 45*, 410-412.

Hazeltine, W. (1993). *Distribution of the scarab beetle* Polyphylla barbata *Cazier* (Unpublished report). Oroville, California.

Hazeltine, W. (1994). Comments for the U.S. Department of Interior, Fish and Wildlife Service, regarding the proposed listing of three species of insects as endangered. In U.S. Fish and Wildlife Service, U.S. Department of Interior (Ed.).

Jones, C.G., Firn, R.D. (1978) The role of phytoecdysteroids in bracken fern,*Pteridium aquilinum* (L.) Kuhn as a defense against phytophagous insect attack. *Journal of Chemical Ecology.* 4(2),117-138.

Lee, D. (1994). *Management proposal for the inland sandhill habitats of Santa Cruz County, CA.* (Unpublished master's thesis) San Jose State University, San Jose, CA.

Loeuille, N., Loreau, M., & Ferriere, R. (2002). Consequences of plant-herbivore coevolution on the dynamics and functioning of ecosystems. *Journal of Theoretical Biology, 217*, 369-381.

Marangio, M. S. (1985). *Preservation study: Sandhills biotic communities of Santa Cruz County, California.* (Unpublished master's thesis) University of California, Berkeley.

McGraw, J. M. (2004). The *Sandhills conservation and management plan: a strategy for preserving native biodiversity in the Santa Cruz Sandhills.* Santa Cruz, CA: The Land Trust of Santa Cruz County.

Nosil, P. (2002). Transition rates between specialization and generalization in phytophagous insects. *Evolution, 56*(8), 1701-1706.

Renwick, J. A. (2001). Variable diets and changing taste in plant-insect relationships. *Journal of Chemical Ecology, 27*(6), 1063-1076.

Russell, D. (1994). *Summary of Mount Hermon Beetle surveys and analysis of species distribution* (Unpublished report). Oxford, Ohio: Miami University.

Scholtens, B. G., & Holland, J. (1997). *Distribution and habitat selection of the Lake Huron Locust (Trimerotropis huroniana).* Report to the Michigan Department of Natural Resources.

Scriber, J. M. (2002). Evolution of insect-plant relationships: chemical constraints, coadaptation, and concordance of insect/plant traits. *Entomologia Experimentalis et Applicata, 104,* 217-235.

Singer, M. S., Bernays, E. A., & Carriere, Y. (2002). The interplay between nutrient balancing and toxin dilution in foraging by a generalist insect herbivore. *Animal Behaviour,* 64, 629-643.

Slaney, D.P.,Blair,D. (2000).Molecules and morphology are concordant in discriminating among populations of cave cockroaches in the genus *Paratemnopteryx Saussure* (Blattodea: Blatellidae). *Annals of the Entomological Society of America,* 93(3), 398-404.

SPSS Inc. (1999). SPSS Base 11.0 for Window's User's Guide. SPSS Inc., Chicago, IL.

Sutherland, W. J. (1996). *Ecological Census Techniques: A Handbook.* New York, NY: Cambridge University Press.

Sword, G. A., & Dopman, E. B. (1999). Developmental specialization and geographic structure of host plant use in a polyphagous grasshopper, *Schistocerca emarginata (=lineata)* (Orthoptera: Acrididae). *Oecologia, 120*(3), 437-445.

Thomas Reid Associates (1998). Revised habitat conservation plan in support of the Amended Incidental Take Permit PRT-830417 under section 10 (a)(1)(B) of the Endangered Species Act. Ventura, CA: U.S. Fish and Wildlife Service.

Thompson, J. N. (1994). *The Coevolutionary Process.* Chicago: The University of Chicago Press.

Thomspon, J.N. (1999). The raw material for coevolution.*Oikos, 84,* 5-16.

Tikkanen, O. P., Niemela, P., & Keranen, J. (2000). Growth and development of a generalist insect herbivore, *Operophtera brumata,* on original and alternative host plants. *Oecologia, 122,* 529-536.

Trewick, S. A. (2001). Identity of an endangered grasshopper (*Acrididae: Brachaspis*): Taxonomy, molecules and conservation. *Conservation Genetics,* 2, 233-243.

U.S.D.A. Soil Conservation Sciences (1980). *Soil survey of Santa Cruz County, California.*

U.S. Fish and Wildlife Service. (1997). *Endangered and threatened wildlife and plants; determination of endangered status for two insects from the Santa Cruz Mountains of California*: Federal Register.

U.S. Fish and Wildlife Service. (1998). *Recovery plan for insect and plant taxa from the Santa Cruz mountains in California.* Portland, Oregon.

Watts, S., & Halliwell, L. (1996). *Essential Environmental Science: Methods and Techniques.* New York: Routledge Press.

West, S. A., & Cunningham, J. P. (2002). A general model for host plant selection in phytophagous insects. *Journal of Theoretical Biology,* 214 (3), 499-513.

Young, R. M. (1967). Polyphylla Harris in America, North of Mexico. Part I: The *Diffracta* complex (Coleoptera: *Scarabaeidae: Melolonthinae*). *Transactions of the American Entomological Society, 93,* 279-318.

Young, R. M. (1988). A Monograph of the Genus *Polyphylla* Harris in America North of Mexico (Coleoptera: *Scarabaeidae: Melolonthinae*). *Bulletin of The University of Nebraska State Museum,* 11(2), 1-106.

Appendix A: Vegetation proportions of each burrow plot

Burrow #	*Larval ID*	*Bare ground*	*Ceanothus cuneatus*	*Chorizanthe pungens var.*	*Ericameria ericoides*	*Eriodicyton californicum*	*Eriogonum nudum*	*Lotus scoparus*	*Lupinus chamissonis*	*Mimulus aurantiacus*	*Moss*	*Other*	*Poaceae spp.*	*Pteridium aquilinum*
04-1	-	66	0	0	0	0	1	1	0	26	3	0	0	3
04-2	S672	80	0	0	0	0	0	15	0	0	0	5	0	0
04-3	S669, S671	67	0	0	24	0	0	9	0	0	0	0	0	0
04-4	-	77	0	0	0	0	0	23	0	0	0	0	0	0
04-5	-	62	0	0	0	0	0	10	0	28	0	0	0	0
04-6	S673	40	0	0	0	0	0	0	0	55	5	0	0	0
04-7	-	43	26	0	0	0	0	0	0	5	15	0	0	0
04-8	-	0	0	0	0	0	85	0	0	0	0	0	0	15
05-1	*	0	0	0	0	0	0	8	0	0	0	12	0	80
05-2	*	74	0	13	0	3	0	0	0	0	0	4	0	0
05-3	*	83	0	0	8	0	1	0	0	0	0	0	0	8
05-4	*	73	0	6	0	0	0	21	0	0	0	0	0	0
05-5	*	59	0	25	0	0	0	3	0	0	0	13	0	0
05-6	*	0	0	0	0	0	0	0	0	0	0	2	0	98
05-7	*	0	0	0	0	0	0	0	3	0	0	0	0	97
05-8	*	25	0	0	0	0	0	0	0	0	0	2	0	73
05-9	S692	79	0	0	0	0	0	5	0	0	0	3	0	0
05-10	*	66	0	0	0	0	1	1	0	26	3	0	0	3

- = burrow excavated and nothing found, * = not excavated. Other species include *Heterotheca grandiflora, Gnaphalium spp., Eschoscholizia californica, Hamata spp., Holocarpha spp., Lessingia filaginifolia, Salivia mellifera*

Appendix B: Vegetation proportions of each non-burrow plot

Plot #	*Arbutus menziesii*	*Arctostaphlyos tomentosa*	*Baccharus pilularis*	*Bare ground*	*Ceanothus cuneatus*	*Chorizanthe pungens var. hartwegiana*	*Ericameria ericoides*	*Eriodictum californica*	*Eriogonum nudum*	*Lotus scoparius*	*Lupinus chamissonis*	*Mimulus aurantiacus*	*moss*	*Other*	*Pinus ponderosa*	*Poaceae*	*Pteridium aquilinum*	*Quercus*	*Salvia mellifera*	*Salix*
r051	0	0	10.75	0	0	0	0	0	0	0	77	0	0	0	0	0	12.3	0	0	0
r051	0	0	10.75	0	0	0	0	0	0	0	77	0	0	0	0	0	12.3	0	0	0
r0510	0	0	0	0	0	0	0	39	0	0	0	0	0	0	2	0	59	0	0	0
r052	0	0	0	0	56.25	0	0	0	0	0	0	0	30	0	0	0	0	0	14	0
r052	0	0	0	0	56.25	0	0	0	0	0	0	0	30	0	0	0	0	0	14	0
r053	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0	0	99.5	0	0	0
r053	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0	0	99.5	0	0	0
r054	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	91	0	0
r054	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	91	0	0
r055	0	0	0	85	0	4.75	0	0	2	0	0	0	0	0	2	0	6	0	0	0
r055	0	0	0	85	0	4.75	0	0	2	0	0	0	0	0	2	0	6	0	0	0
r056	0	0	0	25	0	0	0	73.5	0	0.5	0	0	0	0	1	0	0	0	0	0
r057	0	0	0	0	0	0	0	16	0	0	0	0	0	0	1	0	83	0	0	0

r058	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10 0	0
r058	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10 0	0
r059	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
urs051	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
urs051 0	0	10	0	88	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
urs052	0	47	0	16	0	0	0	0	0	0	0	37	0	0	0	0	0	0	0	0
urs053	0	0	0	59	0	0.65	0	0	0	0	8	0	0	0	0	0	32	0	0	0
urs054	0	0	0	0	0	0	0	0	0	0	0	0	21	0	79	0	0	0	0	0
urs055	0	0	0	24	0	0	0	0	0	0	0	0	0	0	0	0	76	0	0	0
urs056	0	0	75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
urs057	0	0	0	67	0	0	0	0	0	0	24	0	0	0	9	0	0	0	0	0
urs058	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
urs059	0	0	0	90	0	0	0	0	0	0	0	0	4	0	0	0	0	6	0	0
urs1a	0	0	0	66	0	0	0	0	0	0	0	1	0	0	0	0	33	0	0	0
urs1b	0	0	0	33	0	0	0	0	0	0	0	34	0	0	0	0	33	0	0	0
urs2a	0	0	0	3.5	0	0	0	0	0	0	0	50.5	0	0	0	0	0	0	0	4 6
urs2b	0	0	0	76	0	0	0	0	2	1	21	0	0	0	0	0	0	0	0	0
urs3a	4	0	0	86	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0
urs3b	52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	48	0	0	0	0
urs3c	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	97	0	0	0	0
urs4a	0	0	43	0	0	0	0	0	0	0	0	0	12	45	0	0	0	0	0	0
urs4b	0	0	0	0	0	0	0	13	17	0	0	0	12	0	0	0	58	0	0	0
urs4c	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0
urs5a	0	0	0	0	0	0	0	0	0	0	76	0	0	0	24	0	0	0	0	0

urs5b	0	0	0	49	0	0	0	0	0	0	27	0	0	0	0	0	24	0	0	0
urs5c	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0
urs5d	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	95	0	0	0
urs5e	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0
urs5f	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Other species include *Hetereotheca grandifolia, Gnaphalium spp, Eschoscholizia californica, Hamata spp.,Holocarpha spp.,Lessingia filaginifolia, Salvia mellifera*

Appendix C: Relevant Field Notes

6/24/03

7:30 PM – 9:30 PM

Future Mining Area

The point where we set up the black light had *Pinus ponderosa* forest. Saw 3-4 males flying low to the ground and crawling on the ground. A fly was or appeared to be harassing (attacking) the June beetles, getting at their legs or undersides, making it difficult for them to move about. June beetles moved on into some bushes or further away. We climbed up hill to light trap, there were 10 or more June beetles at the light. One was trying to copulate with another, unsuccessfully, moved on to try with other beetles. Rachel noticed that the June beetles bring antennae underneath themselves as it to get a sent on the ground or as it were, the sheet beneath them as they move about. June beetle activity became very slow around 9:30PM.

3/24/04

Visit with Marshall Johnson, Fresno. Also Luis Rodriguez and Marshall's wife Lynn.

Met at In and Out burger, headed to farmer's almong orchard off of 41 (south of Fresno) on Elkhorn Road. Farmer has lost 600 trees to beetle grub infestations and thinks he will lose more soon. His neighbor's farm has been entirely lost to these *Polyphylla sabinia* beetle infestations. We dug under 3 trees-2 live trees with good success (~100 grubs) found none under the dead tree. Sifted soil through a turkey cage or pan. Can find similar things for sifting soil at Orchard Supply-most grubs stayed in pan, smaller ones fell through holes.

Brought home many grubs in a Tupperware bucket, they need to be separated to avoid cannibalism ~10 per bucket. They are active and moving around a lot, probably looking for a way out.

Marshall suggested feeding them carrots, a colleague of his has fed them carrots and they survived. He is still setting up lab with ant farms and PVC pipes with plants for the larvae but said he has kept them in buckets like this from Jan to July or June

Also found diptera larva and wasp ~3ft down-large black and yellow hunter wasp.

Field Observations

5/28/04
8:25 PM ~60F
mostly clear, ½ moon
breezy 5-10 knots
Starting on 1st hill where saw June beetles last year. With Andy tonight. At around 8:40 saw males flying about didn't seem to fly for very long once they flew. Followed them around but no signs of female burrows. All flight activity seemed to stop by 9PM. I think I saw them coming up around *Lotus scoparius* on this hillside. Will have to keep eyes open for this. It had rained earlier in the day, still clouds in the air.

5/29/04
very breezy 7mph
58 F
AREA 5 Spot has *Pinus ponderosas*, *Lotus scoparius*, lupine, rattlesnake grass, *Mimulus aurantiacus*, *Arbutus menziesii.* Moon 2/3rds full. Too windy, no beetles seen at this spot.
SEE MAP drawing…..

6/1/04 Full moon! Warm
70s breezy, not strong
got here early walked around, *Microcoryphia spp.* all over the place.
8:15 PM wind is calming down some what. In area 3, *Pinus ponderosa*, *Mimulus aurantiacus*, *Lotus scoparius*, dead grass, Cyprus, oak, blackberry, grass. No June beetles out here or at 1st hill

6/3/04
8PM Area 4
Old road cuts off to hear, saw some flags yellow flag under *Pinus ponderosa* just below 1st hill and road around 1st hill, *Lotus scoparius*, grasses, willow, cottonwood. Silver-leaf manzanita, monkey flower-quite a mix, disturbed area.
66F, falling, clear skies, breeze
8:35 PM, 64F, breeze picking up

I went back to 1st hill, June beetles are abundant! Bats too. Maybe I should just follow the bats. MHJBs hard to follow, done by 9:15PM

6/5/04

8:40PM 60F

with Andy, *Pinus ponderosa* hillside over area 1. Area 1. Very windy elsewhere in FMA tonight. Bats started flying at 8:50PM and were in by 9:10PM. Marked one spot with frequent male visits with a green stick.

6/8/04

with Monica Nanez (Temp 15 C)

thickly overcast, very little wind

started at Area 4, stayed until quite dark, heard only 1 June beetle buzz but nothing further. Some wind, not enough to deter JB flying as compared to other nights.

8:50 PM went to AREA 1, June beetles and bats were out. Witnessed mating of MHJB between 2 *Lotus scoparius* plants. Saw male fly down, female was on surface, she burrowed in as they mated, leaving no trace. Marked spot with two green sticks. Beetles were done flying by 9:10 PM

6/10/04

with Richard Hollow

clear skies, 56F, cold!

8:45 PM 54F

9:20 PM 52F

windy, not too windy

June beetles out but saw no mating

6/12/04

with Nicole Rucker, Jodi McGraw, Bill (Jodi's partner) 60F

very little wind, 8:51 PM beetles started flying

9:50 PM 58F

two matings!

Jodi and Bill went up to AREA 2, saw no beetles, although very still, came back down to first hill.

Nicole spotted beetles mating close to road, 2 males may have dug out female and fought over her. 1 mated, other stayed neared by and maybe repeated

efforts to mate but did not have success. Mating may have lasted 10 minutes or more. After mating, female burrowed in, males may have burrowed in next to female.
2nd mating was late, male bumbling about until found female burrow. Jodi took pictures of burrow, male grabbed female and pulled her out of burrow and proceeded to mate with her. Female tried to burrow in while male was on top of her (she tried 3 different locations) when we turned off flashlights she very quickly burrowed in. I will make sure to have flashlights off while beetles are trying to mate as it seems to disable them in some way. Male ended up sitting on top of soil after female quickly burrowed under again. She was smaller than him, dirty, difficult to distinguish her or her antennae photos should help though.
Marked both locations with green sticks.

6/15/04
with Rachel
GPS Markings:
N37 04 560
W 122 04 142
1st mating observed 6-8-04 in direct line down from *Pinus ponderosa* close to *Lotus scoparius scoparius, Mimulus aurantiacus,* dead grass

038 6-12-04 mating (pictures!)
N 37 04 562
W 122 04 140
690 ft. near *Lotus scoparius,* and CA poppy

039 6-12-04 mating near the road
N 37 04 570
W 122 04 155
686 ft
Very little vegetation

8:25 PM 60F very little wind, perfect night
9:40 PM 58F
2 matings
Beetles came out at 8:55 PM and were in by 9:40PM

Witnessed matings soon after in both cases 2 males were fighting over 1 female. 1 female was on a herby plant (*Gnaphalium*) 2nd was in grasses near other sight sitting close to the road.
Also saw scorpion, ant lion, flies were staying close to some male MH June beetles as they searched for females. Beetles were flying longer- from 8:55 PM to 9:30 or so and may have still been mating when we left.

6/15/04
With Richard Hollow, 8:40 PM 61 F
Thickly overcast, little wind Area 6
Decided to try hillside across from FMA on road up to it-marked by white posts=maybe marks protected area. Lots of crickets out. One *Pinus ponderosa* not too close by. A little wind picking up-not much. Will wait until 9PM or so to see if beetles come out. If not, will move on to first hill. Went to 1st hill around 9PM beetles were out, not very active-no matings seems they were in by 9:10 PM.

6/19/04
8:57 Out 60F
9:22 in 58F
Becky, Jeff, Andy
4 different spots
Andy in AREA 5, heard beetles flying around. Becky below AREA 3 near road-heard nothing. Jeff in AREA 4, saw and heard nothing until he got close to road and AREA 1. Beetles were out in AREA 1 by 8:58 PM-it was warm and still, no wind. Seemed as though less beetles were flying-no matings witnessed was a quiet night, activity really dropped off after 9:10 PM-only a few flying around after until ~9:22 PM. Moon was crescent but not visible from 1st hill.

6/22/04
Wandered over to muddy water patch (dried sludge pond). Found what may have been P. barbata stuck hard and fast in some dried mud. Looked like hairs caked in mud. Not sure how long it would take to dry out like that.
Saw ants trying to carry off beetle abdominal shell, no elytra attached-in same muddy area.
8:15 PM ~59F windy-winds up to 5-6MPH

8:50 PM 58F on hill where Andy heard beetles flying (Central Mound)

6/28/04

8:40 PM completely overcast little to no wind ~59F
9:30 PM ~59F plenty of beetles out but no mating witnessed. No bats out today. Beetles bumbled around. Saw little mouse?? With long tail. Right at dusk.

7/15/04

P.decemlineata beetle grubs at home
Found 1 sick larva sitting on top of soil- it died soon after I found it. All others are healthy and large with new antennae like structures. Don't know if they have been cannibalizing each other but gave them plenty of fresh carrots. Soil very moist so packed it loosely hopefully it'll dry out a little.

7/15/04

8:42 PM Near hill where Andy had heard beetles. AREA 5. Its warm 70F, some wind.
64F at 9:24 PM
Mating over in lower area of AREA 1. Two males fighting over female near monkey flower. Hear quite a few beetles here. Also sage, lupine, poison oak, shrub.

7/16/04

(Back hill)Back in lower part of AREA 1. Warm night, some clouds, beautiful sunset. I am struck by the number of green and healthy plants in the Quarry. Most of monkey flower here is brown.

8:48 PM 70F
9:30 PM 65 F
Observed two males mating ?? Saw one on the ground, thought it strange that antennae were out like a males, a male flew down and they acted like male and female burrowing into earth. When it stopped both just sat on surface and I couldn't figure out if I had missed something or not until both flew off.

7/20/04

Back hill
8:23 PM It's a little cooler ~65F and a little windy (very little) hopefully will not prove to be a deterrent to June beetles.
Out by 8:42 PM
In by 9:11 PM 58F
Saw mating under sage? Its close to Knobcone pine and *Ceanothus*. A fight ensued between two males-as usual. Mated male was exhausted after mating went down with female into ground part way and returned. Other male tackled him as he returned and tried to find female to no avail. Moon ¾ to almost full.

7/22/04
7:55PM Back hill. 64F
Decided to try hillside where Monica and I were a while ago. Not much wind-saw mating near top of hill where saw one under sage (Back hill) This time under buckwheat-spot may be hard to find marked spot double check. Plenty of them out on this hillside all over in grassy/mixed areas. 50F in by 9:14PM—they were out at 8:46 PM.

7/26/04
Scrub Oak Hill, ¾ moon
8:18 PM 58F. In AREA 4 again-was very windy when I was here 1st time, thought I would try again. Saw mating-other side of drainage ditch (renamed scrub oak hill), near little buckwheat, monkeyflower-dead unidentifiable shrub. Large shrubby area not far from 1st hill. Male June beetles all over this hill in dense thicket areas as well. No *Pinus ponderosa* close by (addendum: 6/28/05- there is immature (8ft tall) *Pinus ponderosa* on other side of hill overlooking quarry pit) by 9PM no more activity.

7/27/04
With Jena
8:40 PM 57F
9:00 PM 53F in Area 4, no matings but saw June beetles out.

7/29/04
with Richard, in AREA 3 again
8:30 PM very still-moon full but not visible here yet. Some cloud cover.

62F 8:45PM
57F 9:06PM
Some flying about-not very long flights or searching flights-low activity-no mating.

8/4/04
8:36PM 59F AREA 3
no clouds, no wind, just me.
8:39PM they are out
9:00 PM they are no longer flying 57F
Again flights limited-not a lot of trackable movement. Very quiet activity, maybe more small movements, no matings seen

5/15/05 GRUB DIG
Dug grubs from 1st hill burrows with J. McGraw and Bill, S.Lambrecht, D.Arnold, R OMalley, R. Hollow, A.Winzelberg, J.Casey
1 grub from burrow #1
2 from burrow #2- the larger grubs are not moving much or at all- very inactive-maybe dead?
2 from burrow #3

5/20/05
Redug grubs in containers
#2, not sure if large grub is dead or not
Burrow 3, #1 also large inactive grub-unsure of its state.
All other grubs seem OK

5/25/05
Redug grubs
Burrow #2 29cm- is still OK
Burrow # 3 (1) larger grub that was inactive is dead, put it in alcohol-found fly larvae in here looks similar to mealworm.
All other grubs alive and healthy looking

5/29/05

1PM – 7PM

Dug up scrub oak hill burrow, thoroughly, found nothing except millipede, Jerusalem crickets and bee pupa. Put everything back in a place.

Went back to first hill and dug in not so random location not at burrow location not far from 2nd burrow location on the hill. Lots of *Ericameria* and no other roots immediately near by. Got 5 grubs from this location and one pupa, put the pupa back when done in same location under some sand.

This is an area where I saw many adult males flying on regular basis. Kept 5 grubs, 2 produced frass easily and I transferred them to soil buckets.

5/31/05

Re-dug 4 larva from 5/15/05 all are alive and OK

Plant last 3 from 5/29/05 dig in soil. One larger one did not look well it is dark colored and inactive. Put all containers w/ individual larva outside with more water, to match soil moisture I found them in, quite moist sand.

5/31/05

64 F Set out D. Arnold's traps 8:15PM. It is clear, warm, no wind. If they are out this early this would be the night. Brought traps in at 9:30PM 5 in #8, 0 #3

I am on top of FMA hill going to farside of hill (down from top) at 8:45 PM bugs were out

6/2/05

Dug again at back hill side in lower regions near *Mimulus aurantiacus*- nothing

Back to 1st hill, dug close to 1st random burrow- 2 pupa, near the surface ~2-3 inches and one larva ~15 inches down which did a nice big dropping. Put pupa back in soil and took larva.

Set Arnold's traps @ 8:20 went back to far side of hill

Breezy ~4mph, cooler than other night (5/31). Temp 15 C

1st mating 9:10-9:15 PM on rocky surface marked with green sticks –later pink flag and GPS'd

#73

N 37 04 599, W 122 04 245 elev. 730ft

Female had difficult time digging in, after a few tries she did get in again but after more than 15 minutes of digging. I faced fierce competition from bats tonight they snapped at all June beetles I was watching and luckily didn't get them all.

6/4/05

with Andy

Dug again near 1st and 2nd random digs no June beetle larva. Found moth pupa and fly larva.

Set out Arnolds traps 8:15PM in by 9:45 PM 4 at #3, 2 at #8

Far side of FMA again

Breezy, cold 50's (12 C) with wind chill. But clear no clouds in sight.

9:10 PM mating female and male got covered with ants. Not sure if ants were attacking or not she managed to burrow in after some time

GPS # 74 N 37 04 528, W 122 04 245 elev. 744ft close to 6/2/05 burrow on same hill top. Noticed pumping action of males while resting – flight warm up?

6/7/05

Dug near other sites again on 1st hill. Just below spot where got 4 grubs, got 1 grub about 2ft deep. Put it in a vial in my pocket and it produced quite a bit of frass. A little cloudy, breezy gusts up to 3mph. On hill near back hill (just before it on the trail up to it) Set out Arnold's traps by 8:15 PM. Saw no June beetles flying near back hill

Traps – 4 in #3, 1 in #8 in by 9:30 PM

No wind on hill where Arnold's traps were.

6/13/05

Was warm today-some high clouds ½ moon but good night I think

set out D. Arnold's traps 8:15 PM

Jodi McGraw is supposed to join me-

Back on far hill down below rocky flat top area- lots of JUNE BEETLES out at 9PM died down by 9:15 PM. Saw mating again by *Ericameria* marked with flag and GPS

GPS #75 N 37 04 515, W 122 04 258 elev. 706 ft

Traps 11 at #3, 15 at #8 in by 9:45 PM

6/15/05

A little cloudy ~30-40% Temp 14 C
Calm no wind yet at 8:25 PM
With Jodi
Arnolds traps out 8:15 PM
Back to far side area
Lots of bats and beetles, bats swooping down to the ground to eat beetles.
Mating – waypoint #76 N 37 04 514, W 122 04 273, elev 644 ft. 1st heard beetles at 8:55PM
Saw mating near some *Chorizanthe*, 4 males fighting over female. Flies were crawling all over the June beetles on their abdomens and just hovering around them. Male stayed on top of female as she burrowed in. Jodi took pictures. All action stopped by 9:15 or so.

6/18/05
With Andy. It rained yesterday and night before so didn't go out. Ground is still wet tonight. Back at far side of FMA-below area of rocky outcrop. Moon is almost full. Arnold's traps out by 8:15 PM. It is clear no wind, some humidity though. Saw no matings but males flying around in large slide area-somewhat hardened slide area. Males had flies all over them and around them harassing them as they searched for females.

6/20/05
Redug larvae. Found 1 from 6/7/05 dig dead. One from 5/29/05 missing, cant' find it in the dirt. 2 more died. Dug up *P. decemlineata*, 1 left from 4 that had legs taken off for DNA analysis. Put 2 in alcohol for comparison/DNA analysis.

6/22/05
with Rachel
Arnolds traps out 8:10 PM in at 9:50 PM 2 at #3, 10 at #8
Windy and cool but not so much wind on far side of FMA where we were. Saw/heard lots of males flying about in lower portion of this area but no matings seen. Moon rose late.

6/25/05
cool, calm, clear night 60F
Traps out 8:20PM

Back to far side of FMA
Bats out, beetles out and moving fast, wandered farther out to bare side facing W. Ridge saw no activity there-back to lower gulch area-lots of flying but nothing
Traps in 9:50 PM
10 at #3, 3 at #8

6/26/05
Decided to try unknown side hill again. Tried 2x last year with no success. But it is clear, calm night-not too cool they should be out given the conditions.
Arnold's traps out at 8:20 PM
Temp 15 C –Mating! After fighting with 3 males, 2 of which traded places atop the female
#77 N 37 04 677, W 122 04 177 elev. 624ft
marked with flag

6/27/05
Dug at 1st hill today, randomly again 3 places no luck. Got traps out late due to wire connection problems 8:40 PM
Back in area I was in last night except on side overlooking dried up pond. A little breeze, little puffs of clouds in the distance but clear warmish night ~ same as last night. No matings, a few buzzes here and there though.
Traps in at 9:30 PM 1 at #3, 11 at #8

6/28/05
Dug again on back hill near *Mimulus aurantiacus*. Dug quite deep ~ 3 ½ feet deep. Found pupa around 2 ½ feet deep.-surprisingly deeper than other pupa I found.

It's clear, calm-warm. 8:30 PM I'm on the other side of the dried pond I was near last night. Lots of live oak here, yerba santa, monkey flower. I am sitting next to a little bit of *Chorizanthe*. Also some coyote bush, plenty of rattlesnake grass. If they are here they should be out tonight. If bats are any indication they aren't here but we'll see.

Saw many many flying males. No matings but must come back!
Watched 1 male flying late in the night- still flying at 9:30 PM, he was

persistently searching one area-finally dropped down into grass and ended up climbing down a hole but likely didn't mate as he came up again quickly and flew off. Males were flying all along perimeter and in edges of dried pond along grasses that had grown in there searching for females there.

7/5/05
Dug for a few hours (6-8:15) on 1st Hill found nothing except one adult male. He seems soft-soft moist shell and antennae as if newly formed or just moist.
8:50 PM Beetles are out. Temp 16 C
Saw mating on edge of dry pond-hard ground-female burrow is very clear. Two males dug her out with difficulty and she was fought over by several males before going back in. Many males flying along edge of dry pond and hill as well as in grass of the hillside. Most stopped flying by 9:15 PM with a few still meandering about on the hill and along edges of dry pond. It has been clear warm night.
Interestingly-female was larger than the males- I noticed this time although I've seen many that look smaller than the males.
WYPT #81
N3704686 W12204072 elev. 587ft.
Traps- 3 @ 8, 9@3. Took pictures of the burrow.

7/7/05

Dug from 5:30-7:45PM on backhill-no luck. Soil is getting drier, deeper all the time. Now ~ 4ft deep before the same moisture content I was finding them at in June.
Arnold's traps out by 8:15PM Temp 16 C
Mating on dry pond hill
Was 82, marked again as 83
82, N3704691 W12204067 567ft
(use 83)
83 N3704 690, W12204067 588ft

7/10/05
Clear warm night, Temp 21 C. Dug 1hr on 1st hill-big deep hole to no avail. Got traps out late – 8:50 PM due to wire problems. Back at dry pond hill-saw to males flying about, heard female digging and found her with ants crawling

on her, very close to perimeter of pond. It's taking her awhile to dig. Can't really tell what the ants are doing except seem interested in her and are crawling all over her-tiny ants. They may be attacking her-biting at her elytra where they touch the back abdominal segments.
Burrow
WYPT # 84
N 3704683, W12204075 562ft

Saw a *Prionus californicous* longhorn beetle walking around on wall-side of roadway between Dry Pond and North Ridge to the right of the ponds. Stopped by it and heard scratching noise nearby. Found another *Prionus* trying to dig its way out of a burrow within the roadway wall. Outside one must have been seeking the one in the burrow.
Saw an interesting katydid and several toads. One large toad with a smaller one. Katydid was typical of S.Cruz County.

7/11/05

Got traps out early- 8PM Temp 18 C
I am on opposite side of Dry Pond Hillside overlooking more disturbed area and pond still filled with muddy water. Clear, calm warm night- moon is thin crescent. Just a touch of a breeze at 8:35 PM. Saw very quick mating! Done in about 1 minute-unusual in that the male didn't have to fight off other males. It's under bracken fern and live oak tree. Marked with red flag.
WYPT #85
N3704692, W12204046 elev. 619ft
Traps in at 9:40PM

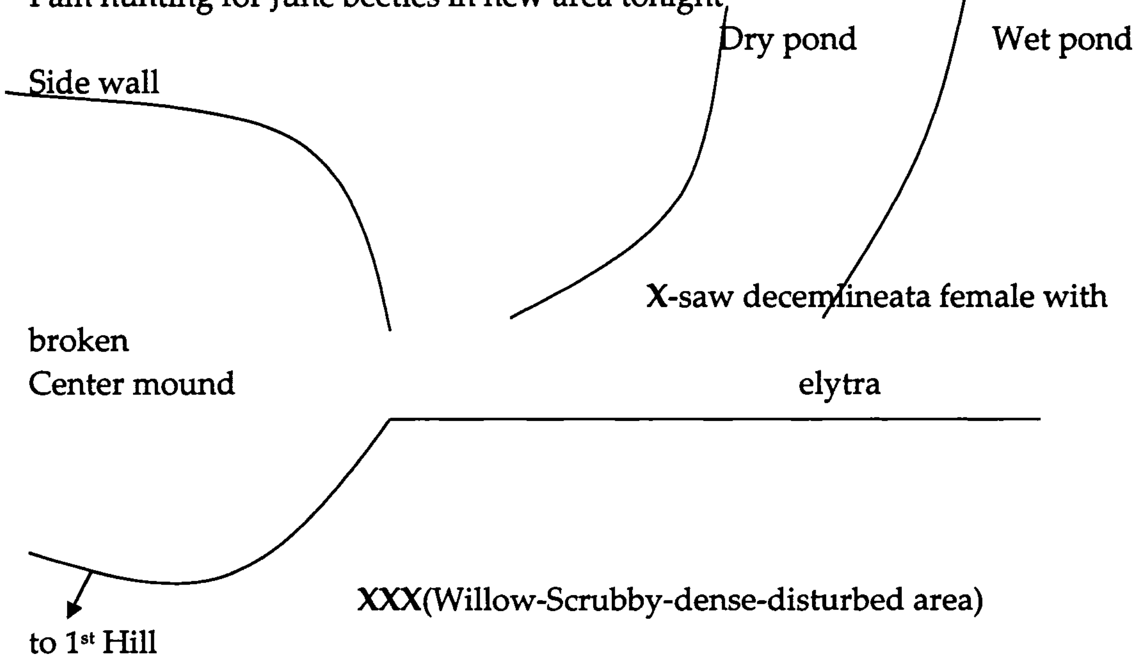
7/12/05
with Jodi McGraw
Far side of FMA is silver-leaf manzanita mixed chaparral with ponderosa
1st hill is degraded parkland
Backhill is degraded parkland
Dry pond hillside is a mélange and degraded parkland.

Am on Wet Pond side of dry hillside with Jodi, Temp 14 C
Another mating-more ants seem to be really tackling (?) June beetles this

time-close to bracken fern mating site. Marked with red flag.
WYPT 86
N3704697, W12204050 582ft
Traps in at 9:45 PM

7/18/05

Traps out by 8:15 PM
Saw 2 coyotes hunting in patch of vegetation between roads in the Quarry. One saw me and started to run but went back to spot where partner was leaping in and out of bushes chasing around rabbit or large rodent that it failed to catch. They were OK with me as long as I didn't move.
I am hunting for June beetles in new area tonight



Area is very disturbed with lots of willow, coyote bush, scrub oak, *Mimulus aurantiacus*, dense thickets of willow and cottonwood. I am on a skinny ridge that has some grass, *Ericameria* and holly??
Clear, warm night did not hear anything on skinny ridge so wandered through scrub listening until reached open area near Monterey pine at parking lot end of scrub oak hill. There are June beetles in this area with

grass, *Ericameria* and *Lotus scoparius*. Full moon! Beetles don't seem bothered by it. No matings but must come back. Saw 1st P. decemlineata female I've seen out here (QHQ) on way down close to where I had started but on the road-side. She is huge! And so different- 2x the size of MHJB males. Her elytra are damaged as may be her wings; she was buzzing about on the ground. Also saw bullfrog leaping about out of one of the series of ponds. It was 8in long, 3-4 in wide. Sort of deflated and hunkered down when I caught up to it and just let me measure it.

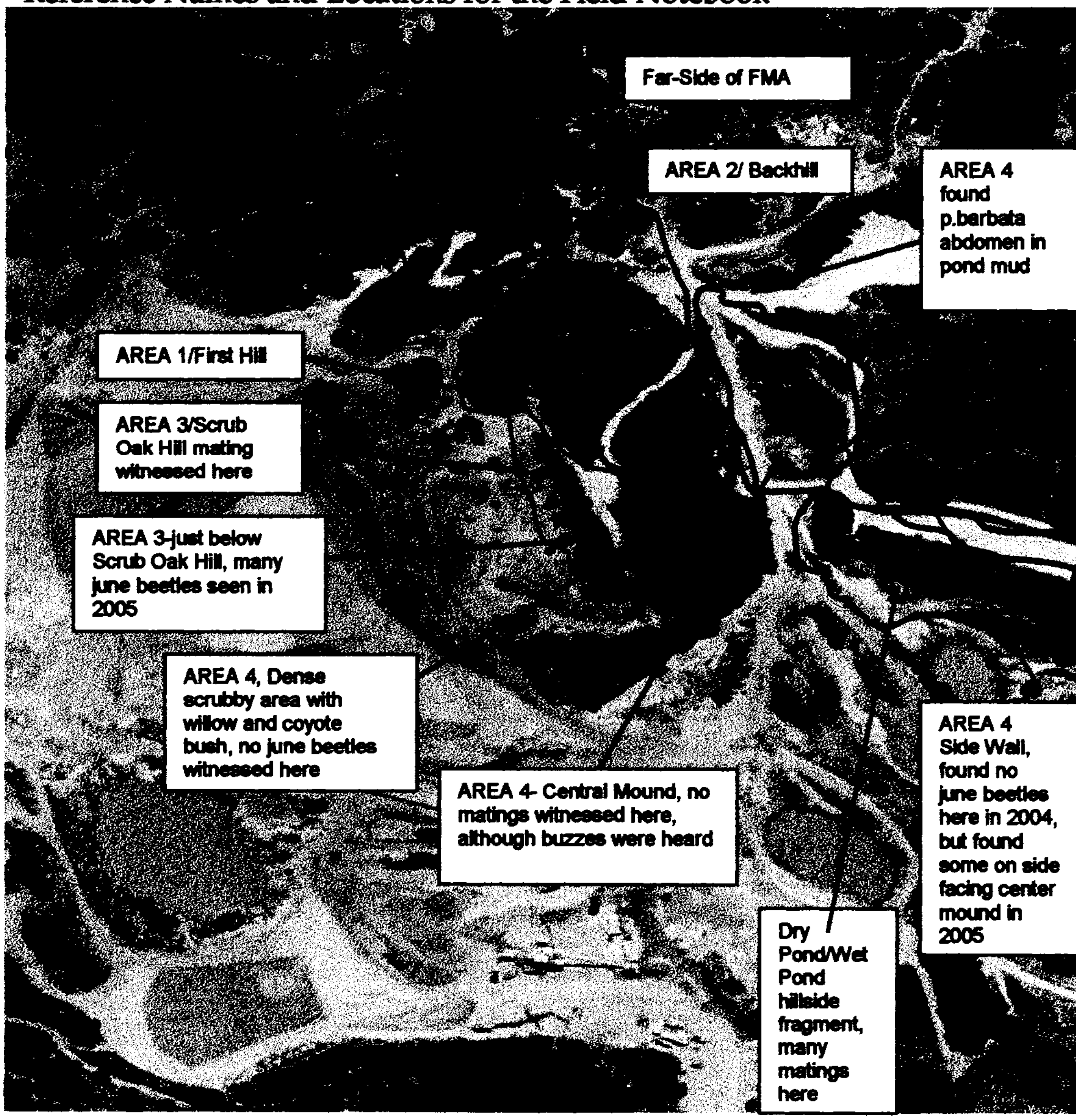
7/20/05
Warm clear night-cooler than previous night nowind
Traps out at 8PM
In area below scrub oak hill overlooking quarry near Monterey pine where I saw June beetles the other night. Yerba santa, *Ericameria, Lotus scoparius,* grass, *Heterotheca grandifolia* are all here and scrub oak and 1 bush of pampas grass. Males are out but difficult to follow –they fly into areas crawl around and leave again.
Traps in 9:50PM

7/21/05
with Jodi McGraw
Same area again as last night. Males out but same sort of activity-searching for females but not finding them. Found dead female close to the Knobcone (previously mislabeled by me as Monterey) pine.

9/14/05
ENVS 185 class came to QHQ and dug burrow 05-10 on Wet Pond Overlook. We found 1 grub about 2ft down.
This grub was later id'd as *P.barbata.* In the frass was identified *Pteridium aquilinum*

9/21/05 *P. sabinia* (or *decemlineata) emerged as adults!*
Larvae collected on 3/24/04 have now emerged.
Over this summer I left the container outside adding little moisture and food infrequently
This may have been the queue they needed to pupate.

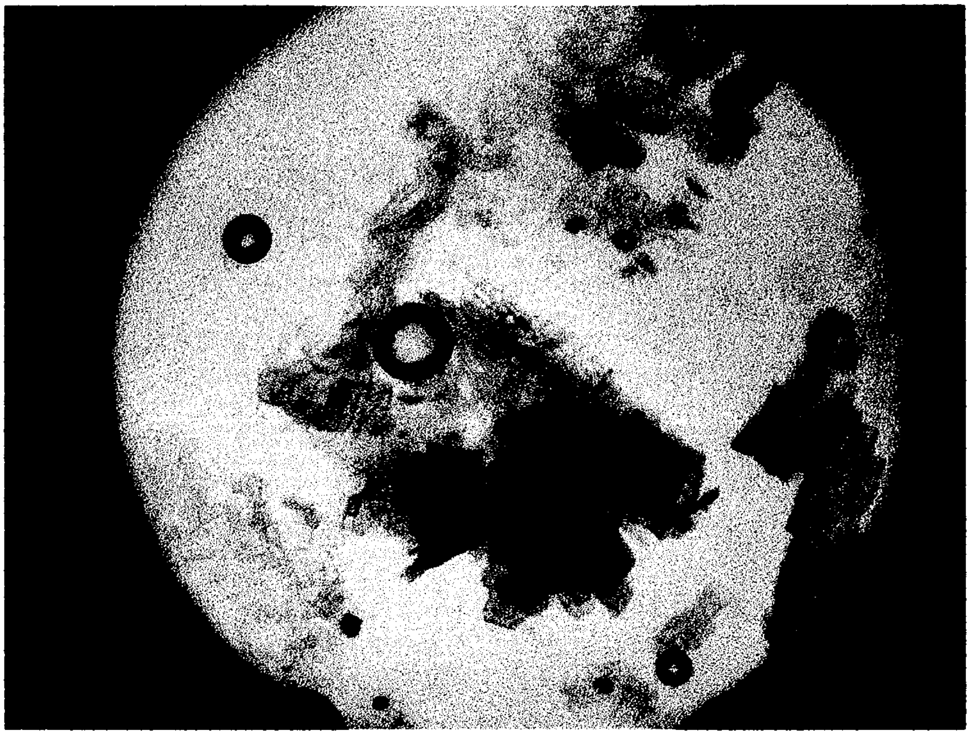
Reference Names and Locations for the Field Notebook

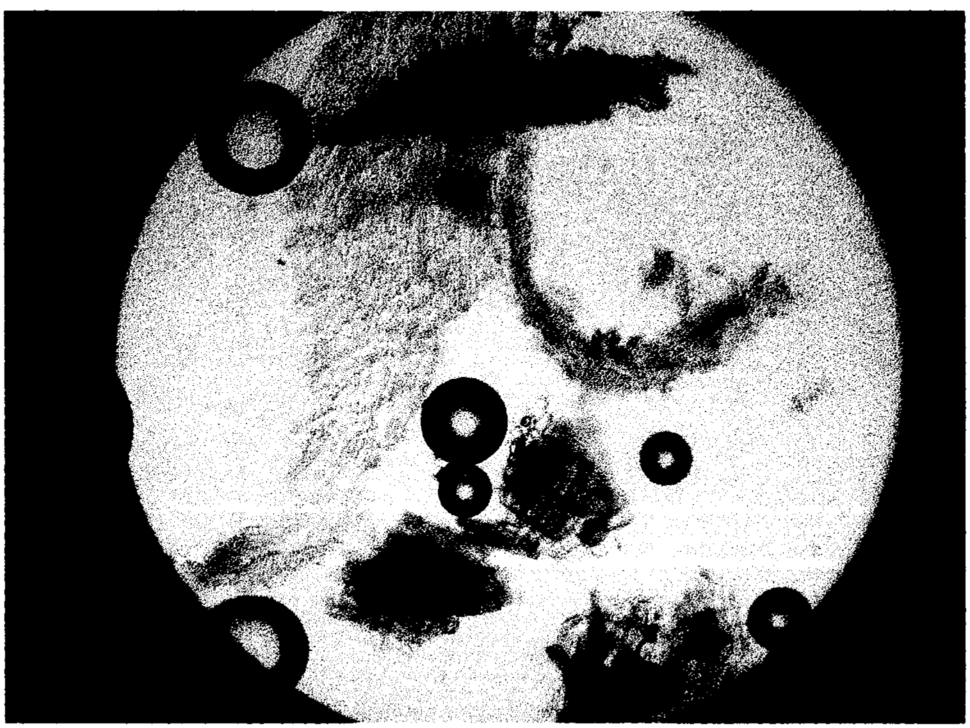


Appendix D: Microscopic photos of plant root tissues [100x].

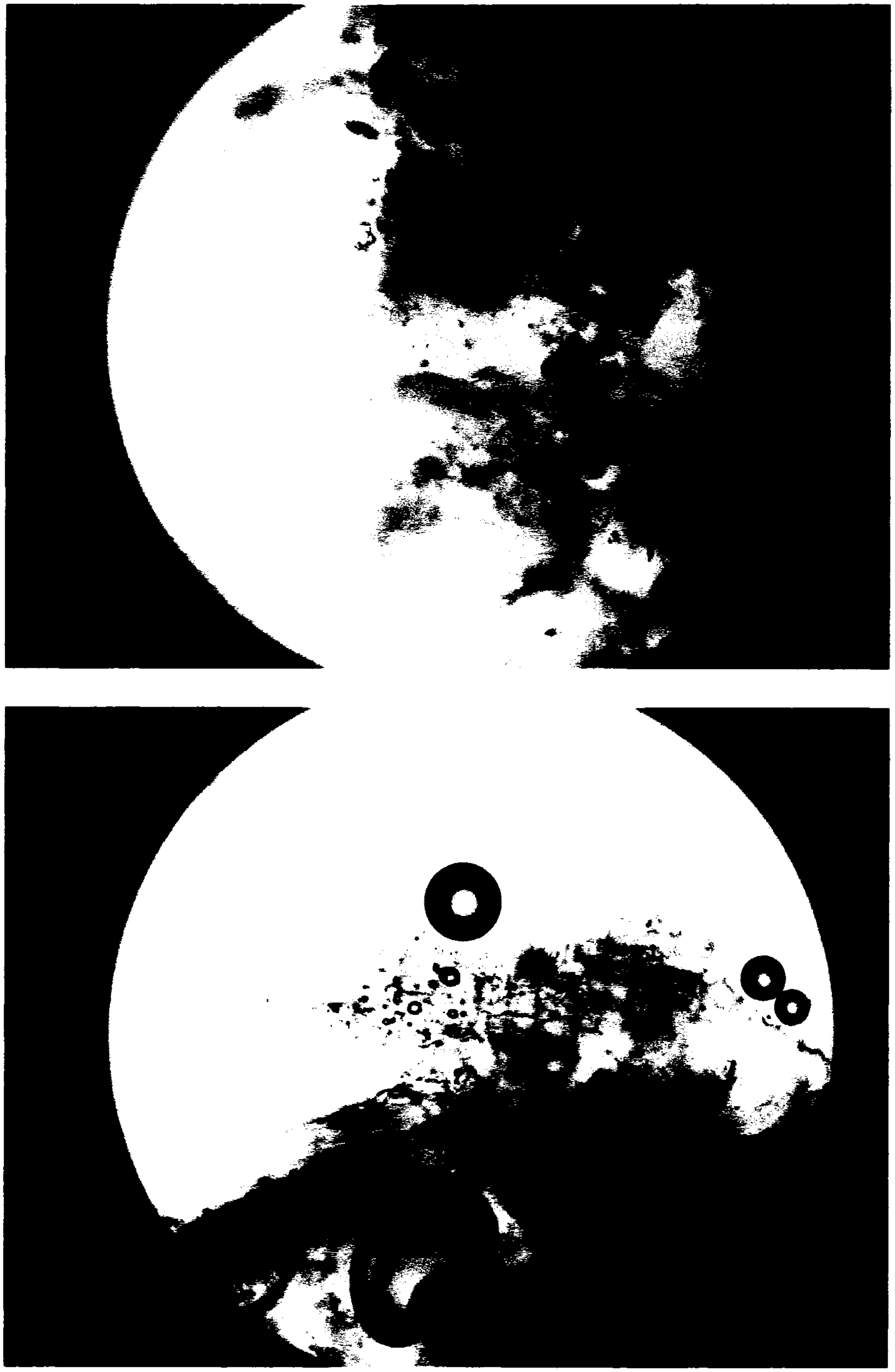
Ericameria ericoides

Eriogonum nudum

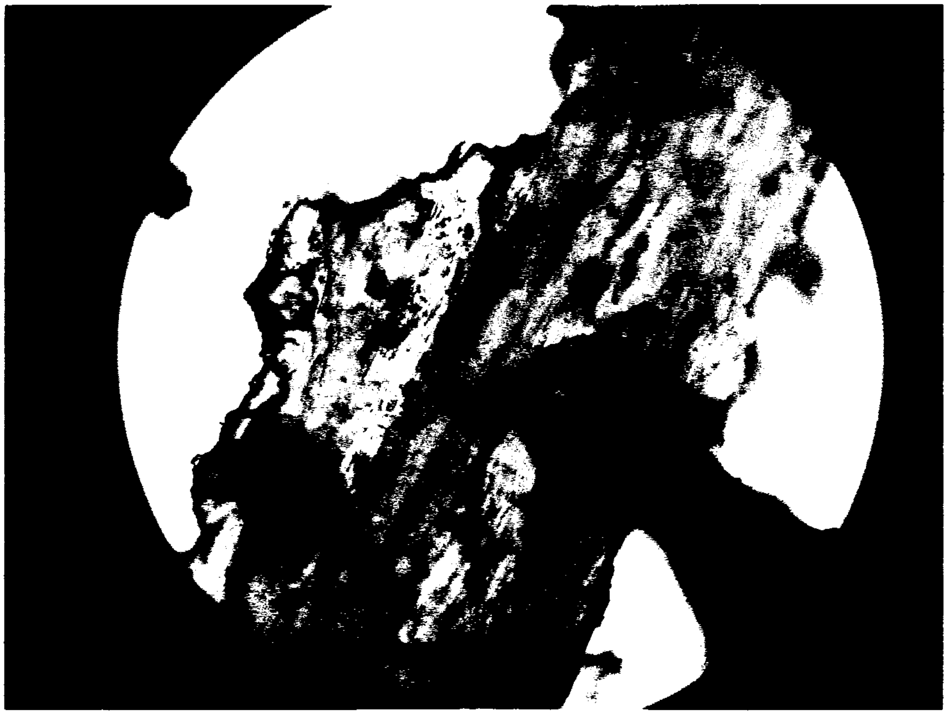


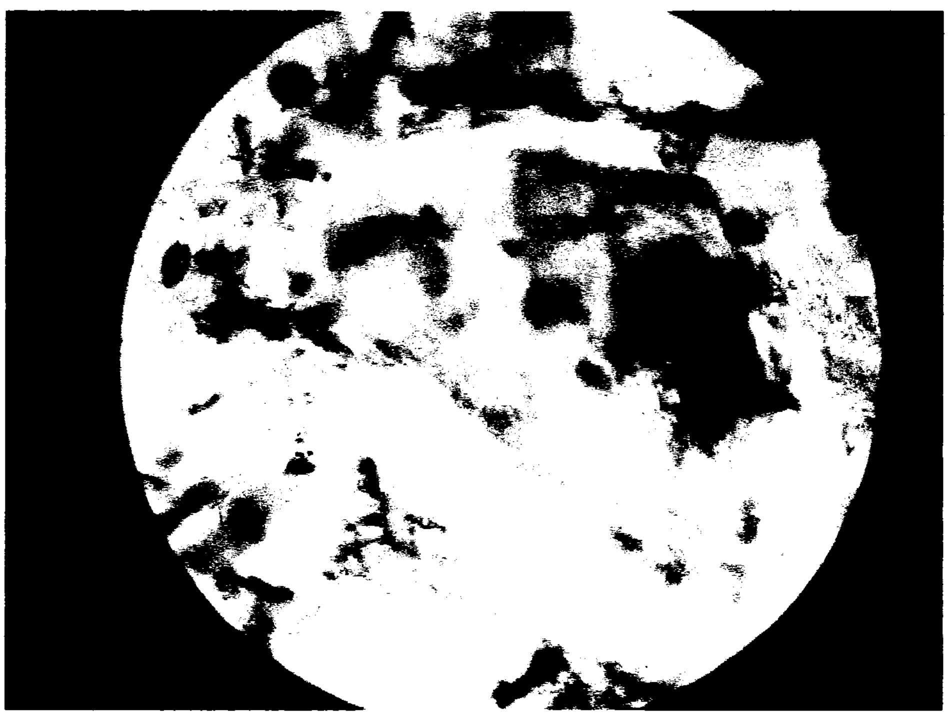


Escholitzia californica

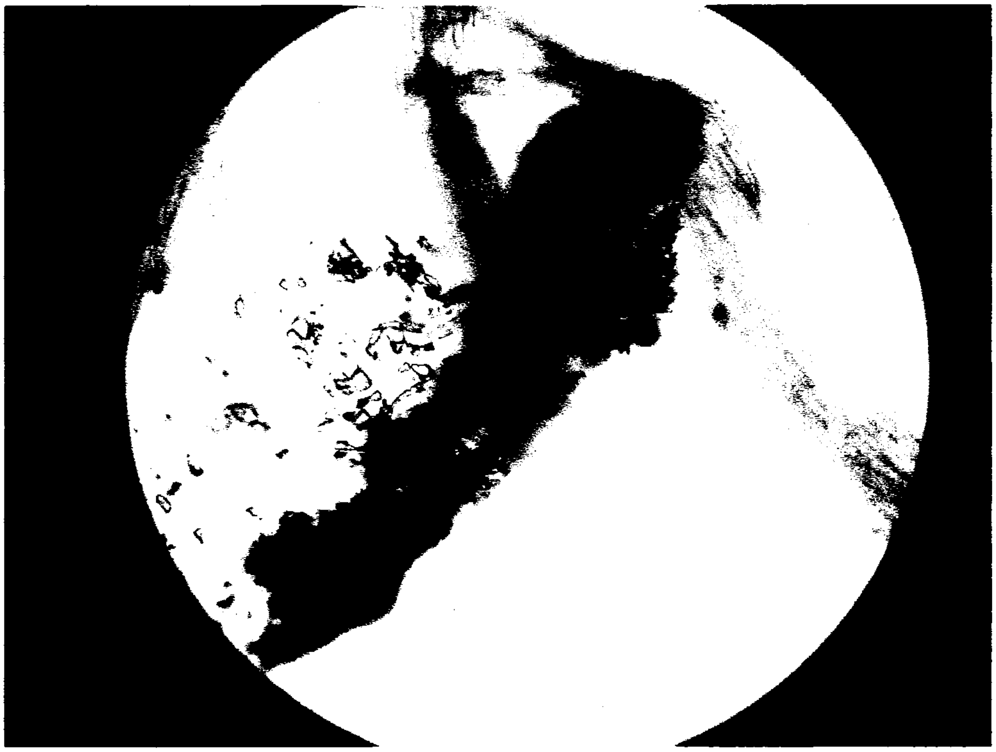


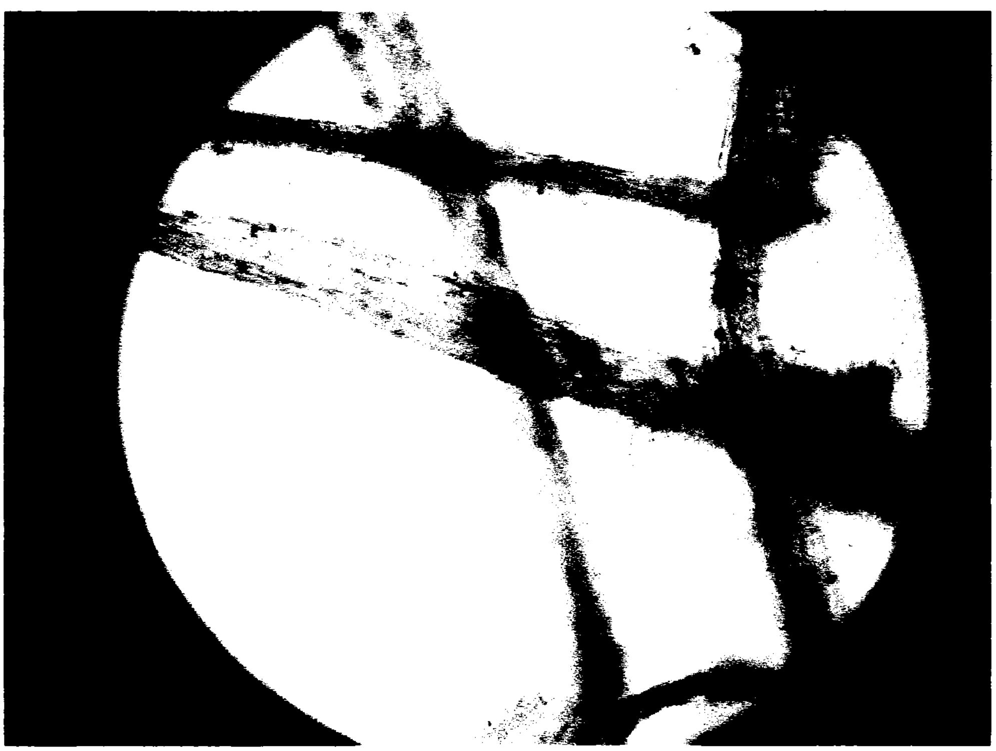
Heterotheca grandifolia



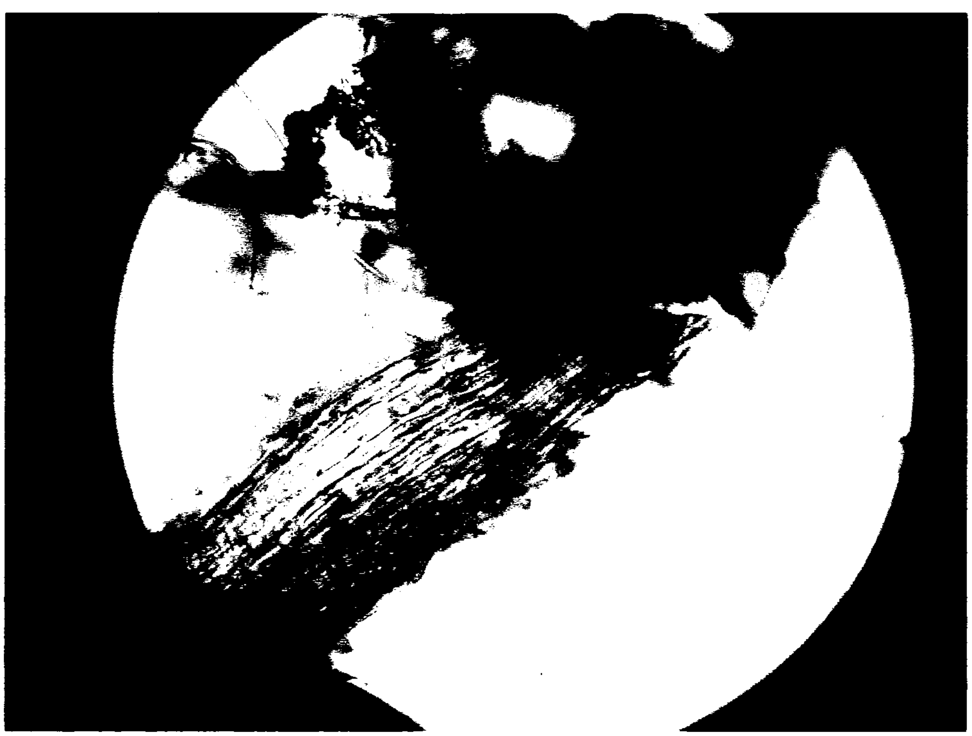


Lessingia filaginifolia var. filaginifolia

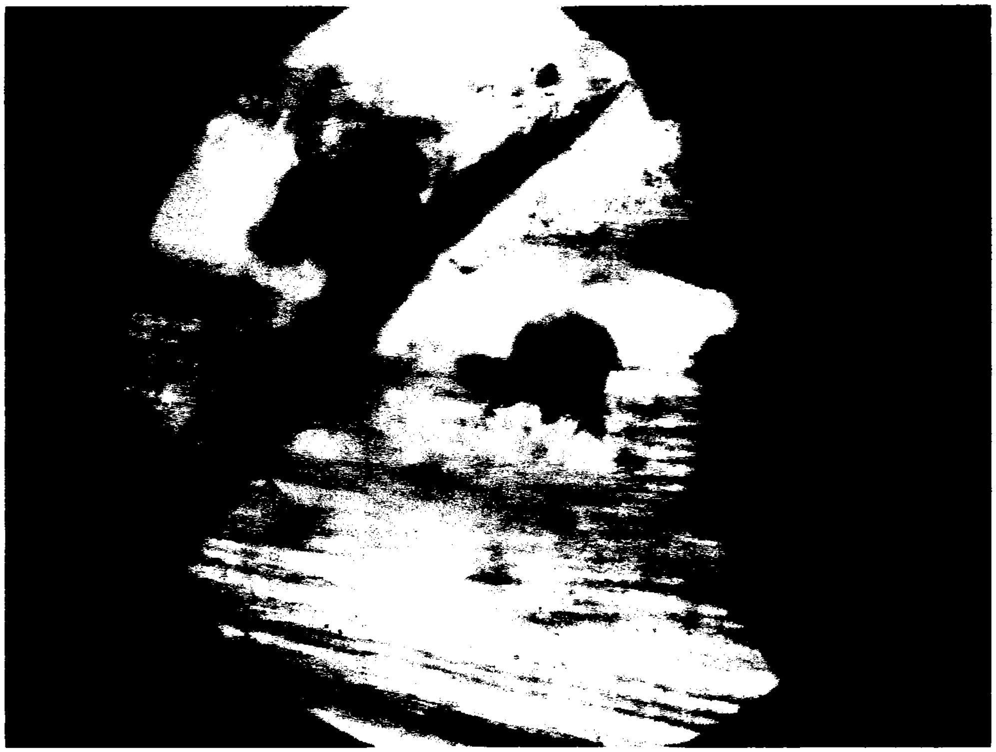


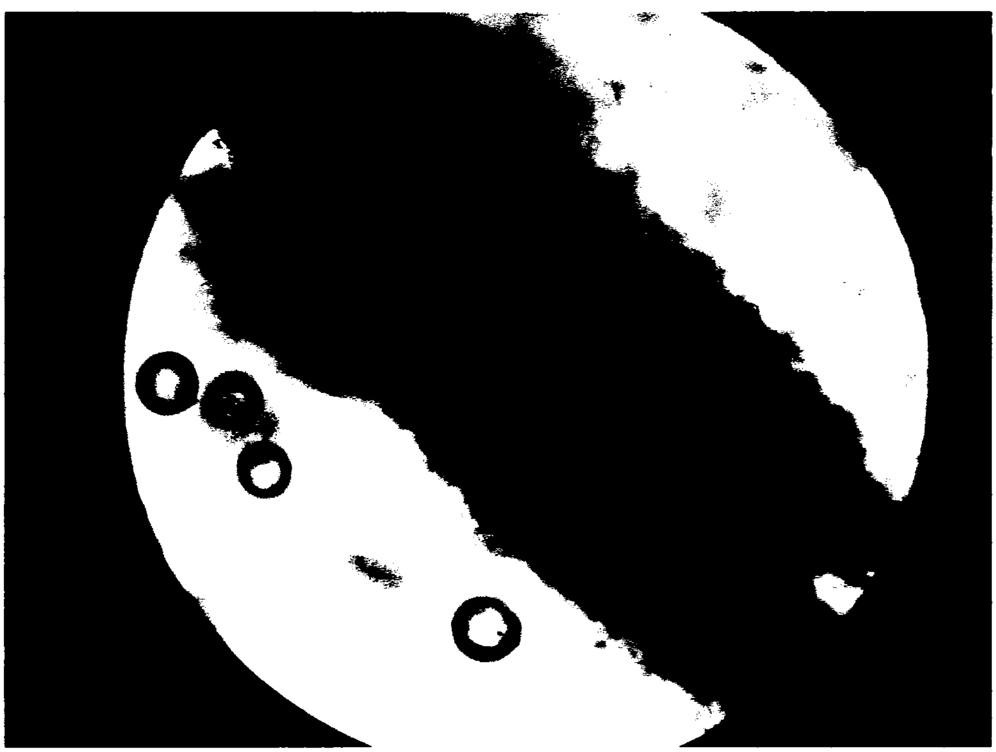


Lotus scoparius

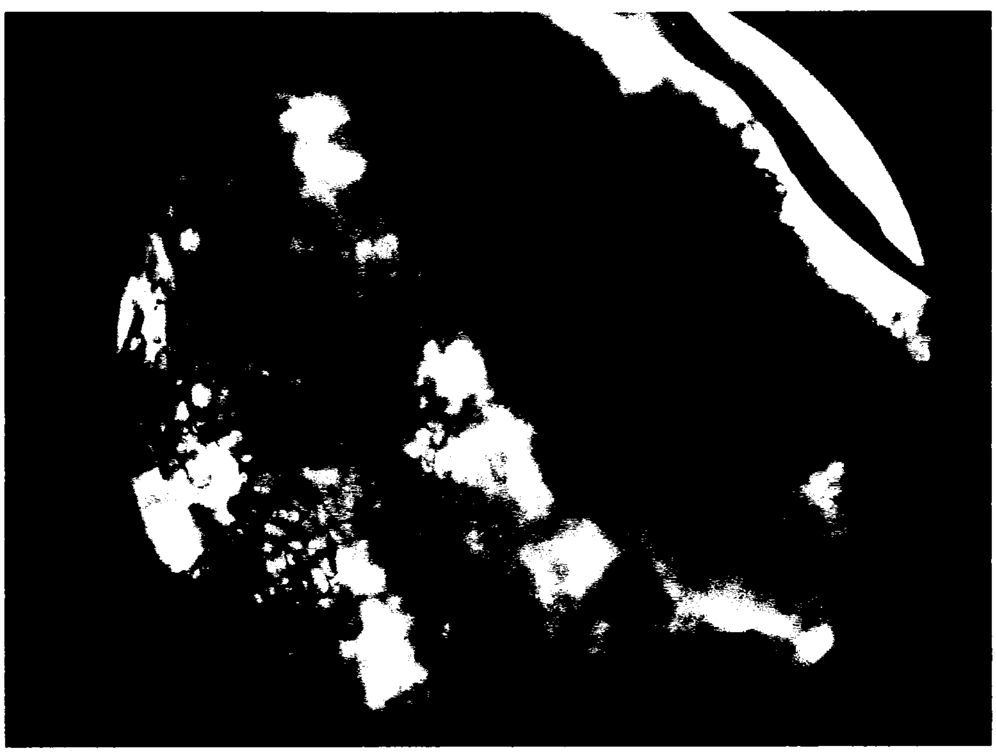


Lupinus chamissonis

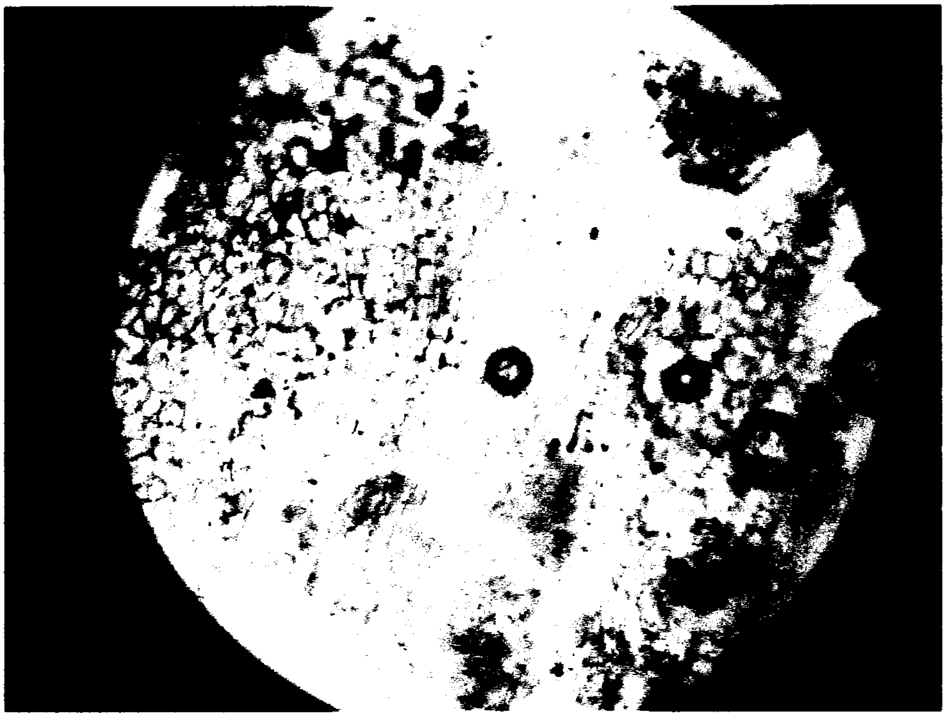


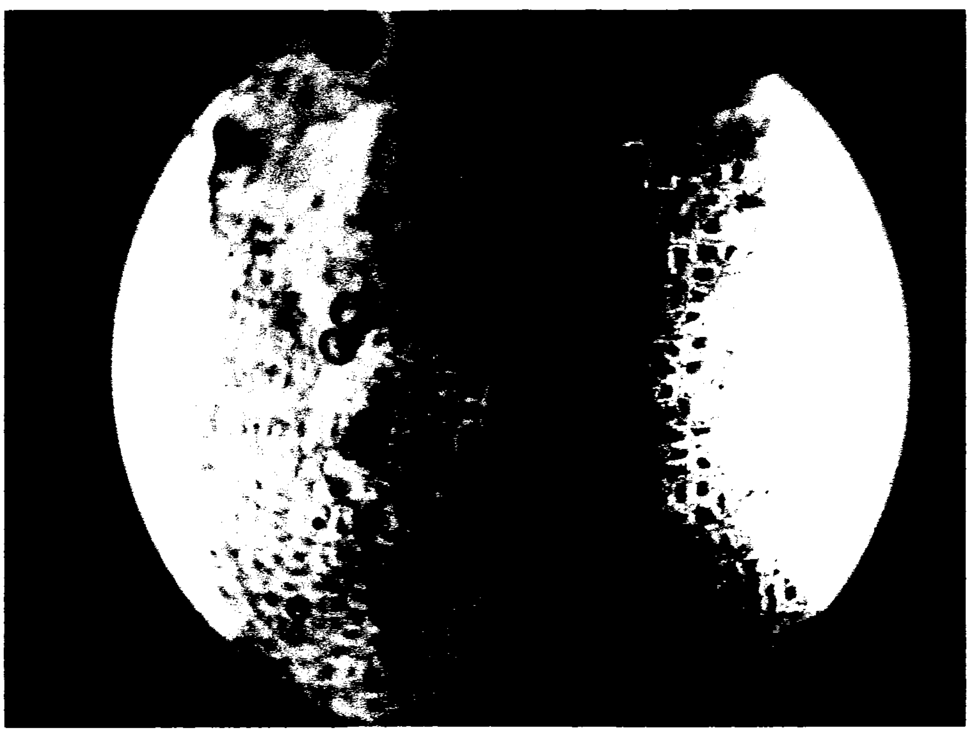


Mimulus aurantiacus

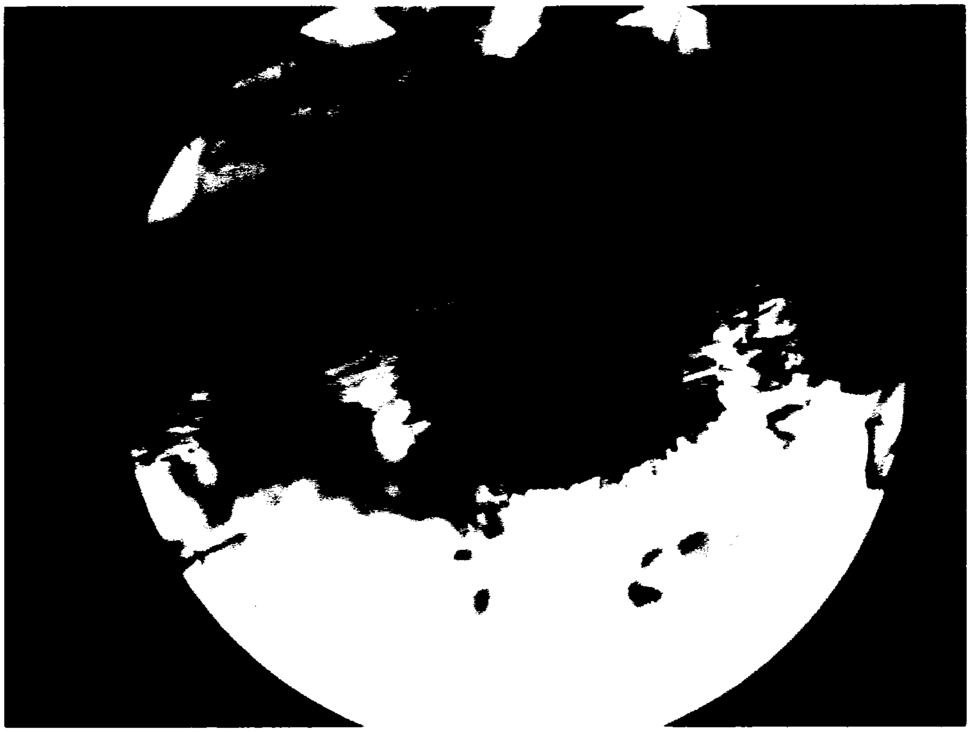


Pinus ponderosa



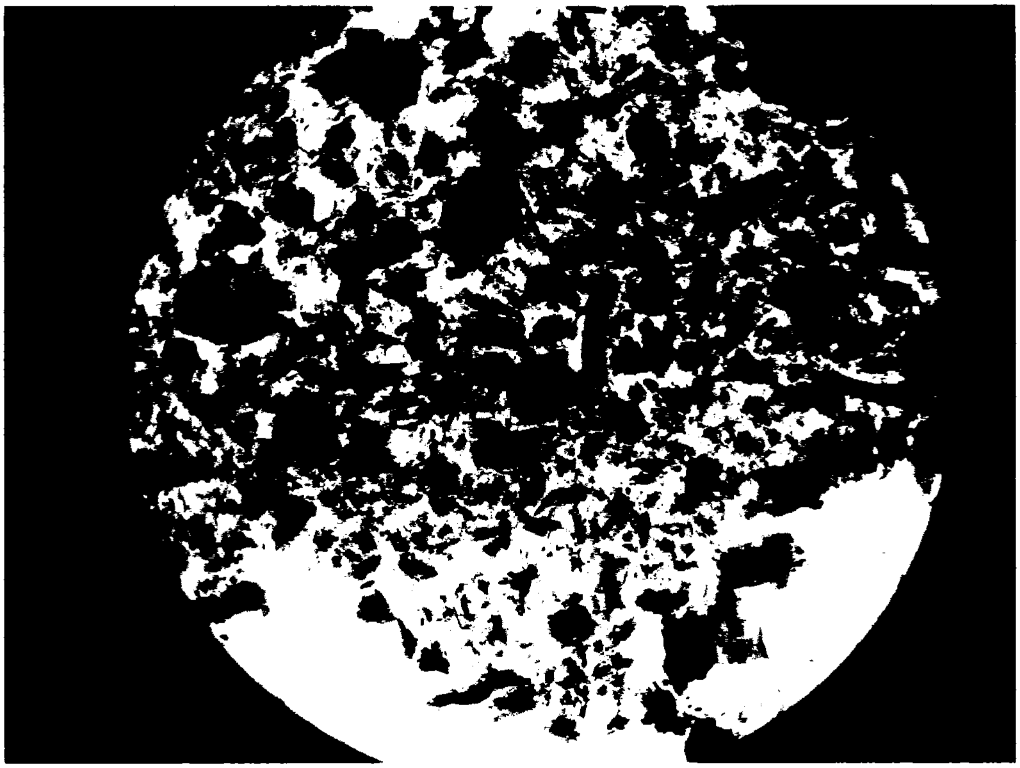


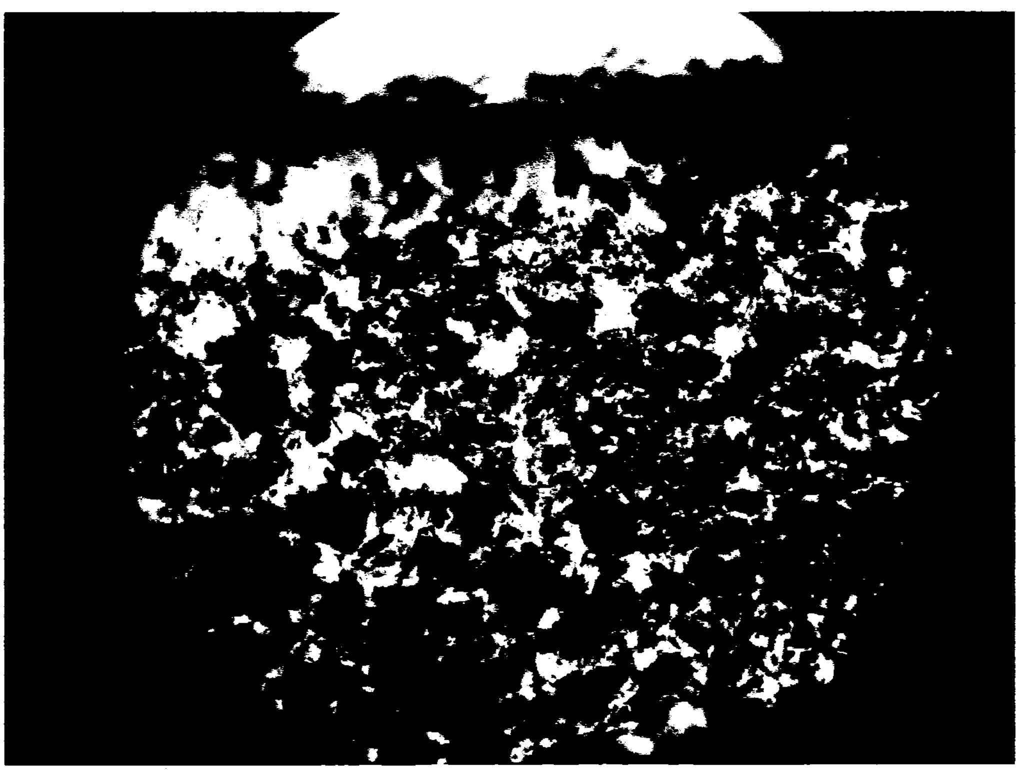
Pteridium aquilinum



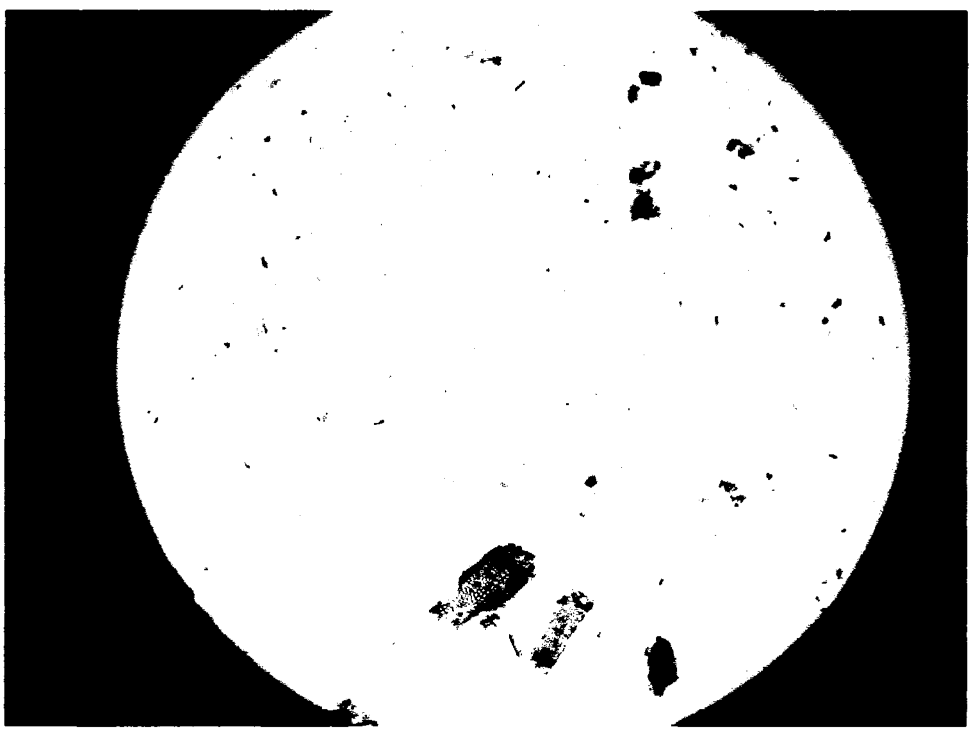
Appendix E: Microscopic photos of larval frass samples [100X].

Collected: 5/15/05. Larval DNA marker #S673

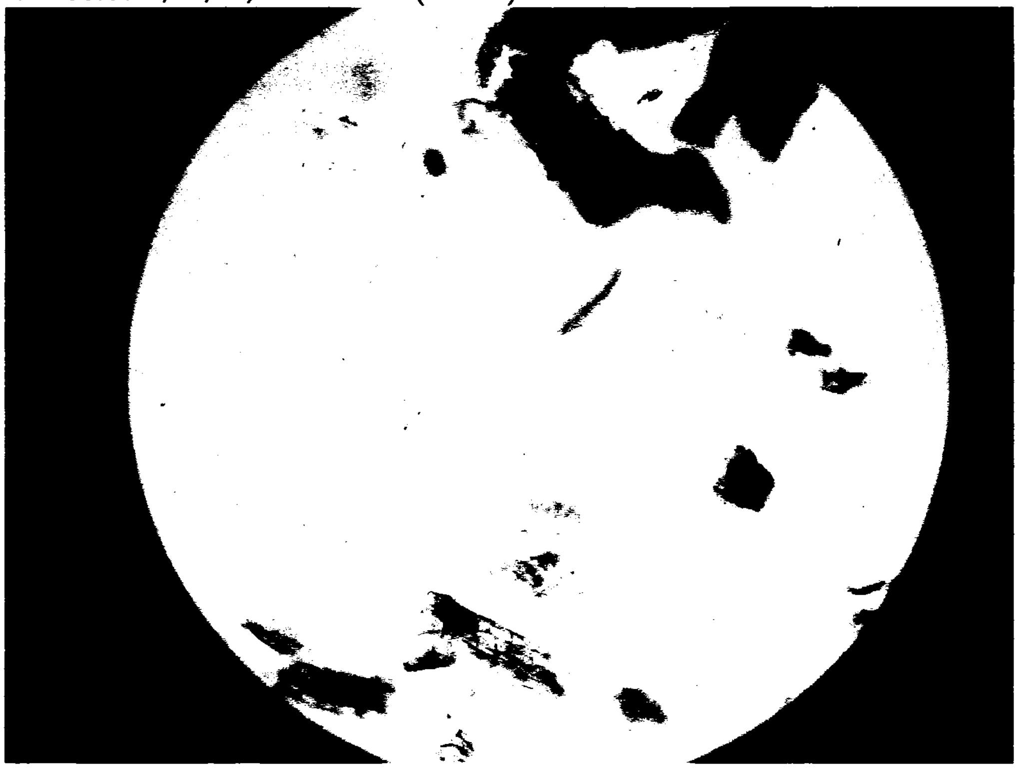


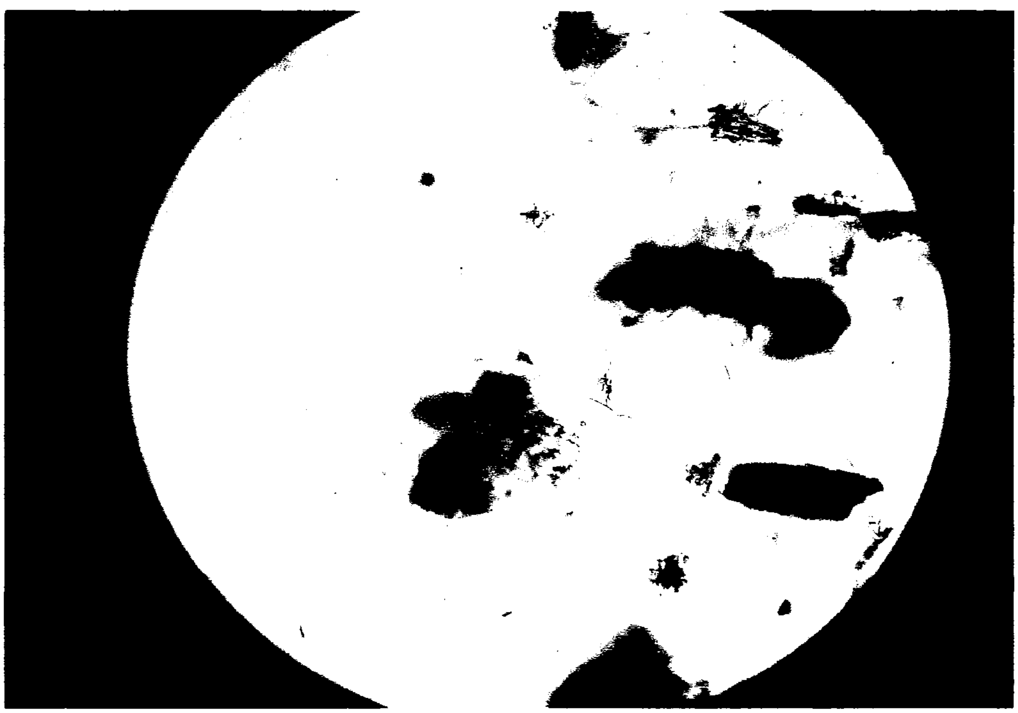


Collected: 5/29/05, Larval DNA marker #S669a

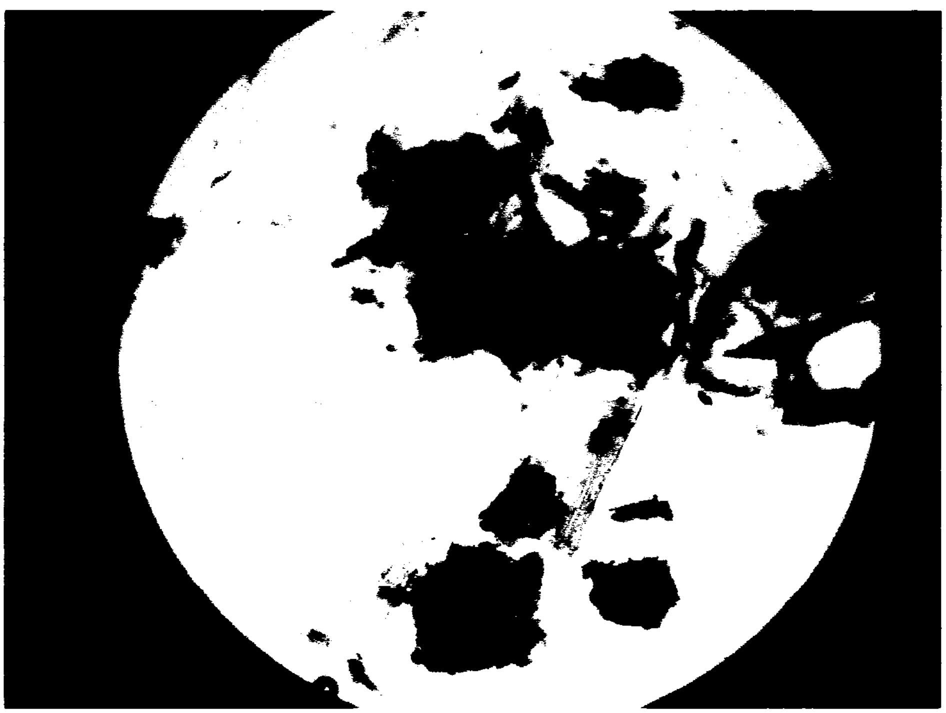


Collected 5/29/05, Unknown (S669b)

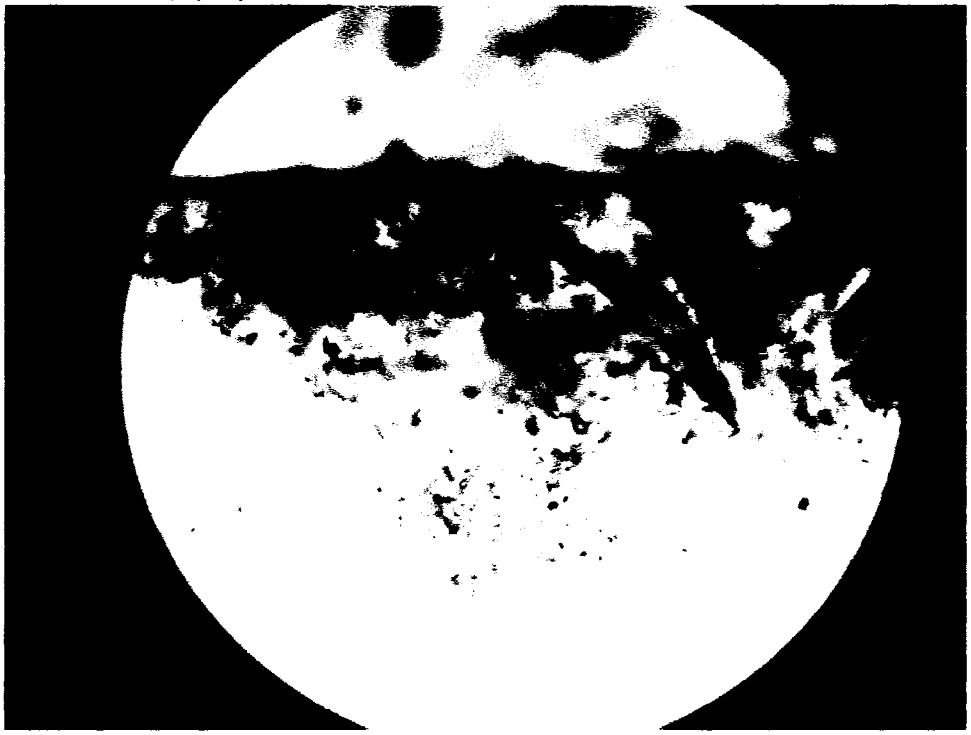


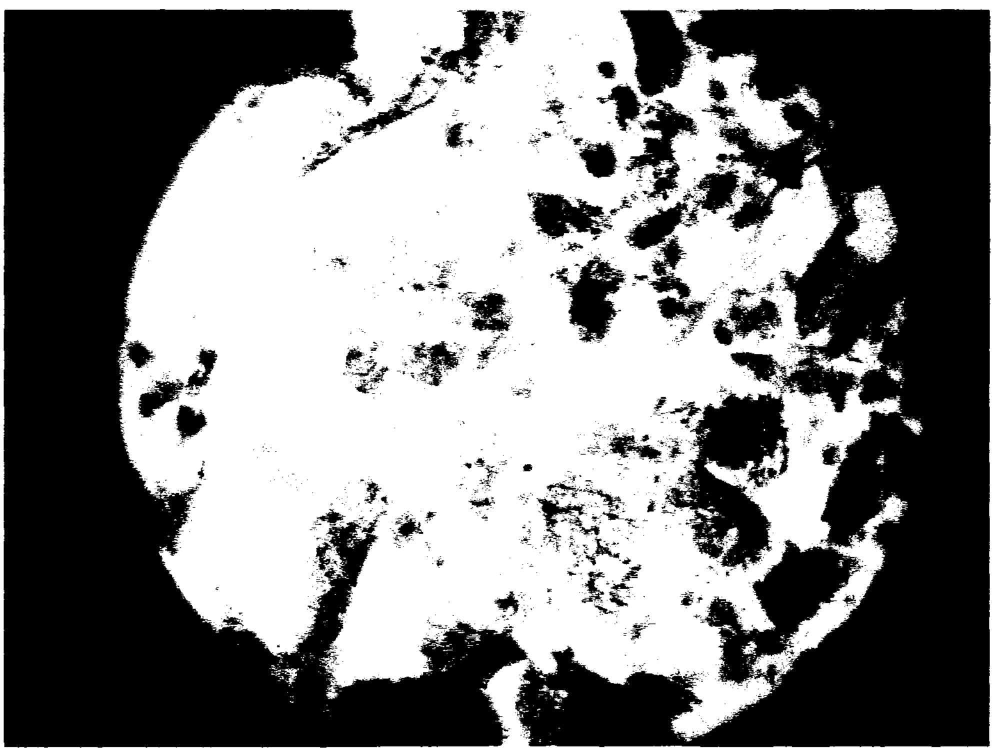


Collected 6/2/05, S672



Collected 6/7/05, Larval DNA marker #S671





Appendix F: DNA sequences for ITS2 gene comparison

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                      10        20        30        40        50        60
                       .         .         .         .         .         .
Polyph.decem.S675   TTGCGGTCCTCGGATACTGTTCCCGGACCACTCCTGACTGAGGGTCGTTTTGATGTACAC
Polyph.decem.S685   TTGCGGTCCTCGGATACTGTTCCCGGACCACTCCTGACTGAGGGTCGTTTTGATGTACAC
Polyph.barbat.S674  TTGCGGTCCTCGGATACTGTTCCCGGACCACTCCTGACTGAGGGTCGTTTTGATGTACAC
Grub1.S669          TTGCGGTCCTCGGATACTGTTCCCGGACCACTCCTGACTGAGGGTCGTTTTGATGTACAC
Grub3.S671          TTGCGGTCCTCGGATACTGTTCCCGGACCACTCCTGACTGAGGGTCGTTTTGATGTACAC
Grub4.S672          TTGCGGTCCTCGGATACTGTTCCCGGACCACTCCTGACTGAGGGTCGTTTTGATGTACAC
Grub5.S673          TTGCGGTCCTCGGATACTGTTCCCGGACCACTCCTGACTGAGGGTCGTTTTGATGTACAC
Grub6.S692          TTGCGGTCCTCGGATACTGTTCCCGGACCACTCCTGACTGAGGGTCGTTTTGATGTACAC
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                         70        80        90        100       110
                         .         .         .         .         .
Polyph.decem.S675   AACTGCGCGGCGTG[illegible]CGAATCATTCTCGTCGTGCCGTGTTCTAAACGGCGTCAAGGCGT
Polyph.decem.S685   AACTGCGCGGCGTG[illegible]CGAATCATTCTCGTCGTGCCGTGTTCTAAACGGCGTCAAGGCGT
Polyph.barbat.S674  AACTGCGCGGCGTG[illegible]CGAATCATTCTCGTCGTGCCGTGTTCTAAACGGCGTGAAGGCGT
Grub1.S669          AACTGCGCGGCGTG[illegible]CGAATCATTCTCGTCGTGCCGTGTTCTAAACGGCGTGAAGGCGT
Grub3.S671          AACTGCGCGGCGTG[illegible]CGAATCATTCTCGTCGTGCCGTGTTCTAAACGGCGTGAAGGCGT
Grub4.S672          AACTGCGCGGCGTG[illegible]CGAATCATTCTCGTCGTGCCGTGTTCTAAACGGCGTGAAGGCGT
Grub5.S673          AACTGCGCGGCGTG[illegible]CGAATCATTCTCGTCGTGCCGTGTTCTAAACGGCGTGAAGGCGT
Grub6.S692          AACTGCGCGGCGTG[illegible]CGAATCATTCTCGTCGTGCCGTGTTCTAAACGGCGTGAAGGCGT
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                  120       130        140        150        160       170
                   .         .          .          .          .         .
Polyph.decem.S675 GTCCGCACTGCCAATCCCCGTGCACACGGCAACACGACAGTGAAGGTTCATCGTCGTCG
Polyph.decem.S685 GTCCGCACTGCCAATCCCCGTGCACACGGCAACACGACAGTGAAGGTTCATCGTCGTCG
Polyph.barbat.S674 GTTCGCACTGCCAATCCCCGTGCACACGGCAAC--GACAGTGAAGGTTCATCGTCGTCG
Grub1.S669        GTTCGCACTGCCAATCCCCGTGCACACGGCAAC--GACAGTGAAGGTTCATCGTCGTCG
Grub3.S671        GTTCGCACTGCCAATCCCCGTGCACACGGCAAC--GACAGTGAAGGTTCATCGTCGTCG
Grub4.S672        GTTCGCACTGCCAATCCCCGTGCACACGGCAAC--GACAGTGAAGGTTCATCGTCGTCG
Grub5.S673        GTTCGCACTGCCAATCCCCGTGCACACGGCAAC--GACAGTGAAGGTTCATCGTCGTCG
Grub6.S692        GTTCGCACTGCCAATCCCCGTGCACACGGCAAC--GACAGTGAAGGTTCATCGTCGTCG
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                    180       190       200       210       220       230
                      .         .         .         .         .         .
Polyph.decem.S675   CAAGGGAGACGGAGATCGATGTCACACGGCACCGATCACCGTCCGTCCTGGGAAGTTC
Polyph.decem.S685   CAAGGGAGACGGAGATCGATGTCACACGGCACCGATCACCGTCCGTCCTGGGAAGTTC
Polyph.barbat.S674  CAAGGGAGACGGAGATCGATGTCACACGGCACCGATCACCGTCCGTCCTGGGAAGTTC
Grub1.S669          CAAGGGAGACGGAGATCGATGTCACACGGCACCGATCACCGTCCGTCCTGGGAAGTTC
Grub3.S671          CAAGGGAGACGGAGATCGATGTCACACGGCACCGATCACCGTCCGTCCTGGGAAGTTC
Grub4.S672          CAAGGGAGACGGAGATCGATGTCACACGGCACCGATCACCGTCCGTCCTGGGAAGTTC
Grub5.S673          CAAGGGAGACGGAGATCGATGTCACACGGCACCGATCACCGTCCGTCCTGGGAAGTTC
Grub6.S692          CAAGGGAGACGGAGATCGATGTCACACGGCACCGATCACCGTCCGTCCTGGGAAGTTC
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	240	250	260	270	280	290

Polyph.decem.S675	GAATGTGAATGTGCGCTCGTGTAATTTAATTACACTCGCCACTCGCTCGTTTCCCGAAA
Polyph.decem.S685	GAATGTGAATGTGCGCTCGTGTAATTTAATTACACTCGCCACTCGCTCGTTTCCCGAAA
Polyph.barbat.S674	GAATGTGCAATGTGGCTCGTGTAATTTAATTACACTTGCCACTCGCTCGTTTCCCGAAA
Grub1.S669	GAATGTGCAATGTGGCTCGTGTAATTTAATTACACTTGCCACTCGCTCGTTTCCCGAAA
Grub3.S671	GAATGTGCAATGTGGCTCGTGTAATTTAATTACACTTGCCACTCGCTCGTTTCCCGAAA
Grub4.S672	GAATGTGCAATGTGGCTCGTGTAATTTAATTACACTTGCCACTCGCTCGTTTCCCGAAA
Grub5.S673	GAATGTGCAATGTGGCTCGTGTAATTTAATTACACTTGCCACTCGCTCGTTTCCCGAAA
Grub6.S692	GAATGTGCAATGTGGCTCGTGTAATTTAATTACACTTGCCACTCGCTCGTTTCCCGAAA

	300	310	320	330	340

Polyph.decem.S675	ACATGCTTTTTAGCACGGGACACATAGCCGCGTCATGACGGTTGCAGTAACTGGC
Polyph.decem.S685	ACATGCTTTTTAGCACGGGACACATAGCCGCGTCATGACGGTTGCAGTAACTGGC
Polyph.barbat.S674	ACATGCTTTTTAGCACGGGACACATAGCCGCGTCATGACGGTTGCAGTAACTGGC
Grub1.S669	ACATGCTTTTTAGCACGGGACACATAGCCGCGTCATGACGGTTGCAGTAACTGGC
Grub3.S671	ACATGCTTTTTAGCACGGGACACATAGCCGCGTCATGACGGTTGCAGTAACTGGC
Grub4.S672	ACATGCTTTTTAGCACGGGACACATAGCCGCGTCATGACGGTTGCAGTAACTGGC
Grub5.S673	ACATGCTTTTTAGCACGGGACACATAGCCGCGTCATGACGGTTGCAGTAACTGGC
Grub6.S692	ACATGC TTTTAGCACGGGACACATAGCCGCGTCATGACGGTTGCAGTAACTGGC

	350 . 360 . 370 . 380 . 390 . 400 .
Polyph.decem.S675	GCCCGCGTGCACACACAAATTTCGTCGAAGGTCGAAACTCCTAGTGGGCGACCCGTAA
Polyph.decem.S685	GCCCGCGTGCACACACAAATTTCGTCGAAGGTCGAAACTCCTAGTGGGCGACCCGTAA
Polyph.barba.S674	GCCCGCGTGCACACACAAATTTCGTCGAAGGTCGAAACTCCTAGTGGGCGACCCGTAA
Grub1.S669	GCCCGCGTGCACACACAAATTTCGTCGAAGGTCGAAACTCCTAGTGGGCGACCTGTAA
Grub3.S671	GCCCGCGTGCACACACAAATTTCGTCGAAGGTCGAAACTCCTAGTGGGCGACCTGTAA
Grub4.S672	GCCCGCGTGCACACACAAATTTCGTCGAAGGTCGAAACTCCTAGTGGGCGACCTGTAA
Grub5.S673	GCCCGCGTGCACACACAAATTTCGTCGAAGGTCGAAACTCCTAGTGGGCGACCTGTAA
Grub7.S692	GCCCGCGTGCACACACAAATTTCGTCGAAGGTCGAAACTCCTAGTGGGCGACCTGTAA

	410 420 430 440 450 460
Polyph.decem.S675	CGGCTGGTGTGATCTTGCGCCGGGCGTCTGTGAAATTGTAGCATTAATGTAATGAGAG
Polyph.decem.S685	CGGCTGGTGTGATCTTGCGCCGGGCGTCTGTGAAATTGTAGCATTAATGTAATGAGAG
Polyph.barbat.S674	CGGCTGGTGTGATCTTGCGCCGGGCGTCTGTGAAATTGTAGCATTAATGTAATGCGAG
Grub1.S669	CGGCTGGTGTGATCTTGCGCCGGGCGTCTGTGAAATTGTAGCATTAATGTAATGCGAG
Grub3.S671	CGGCTGGTGTGATCTTGCGCCGGGCGTCTGTGAAATTGTAGCATTAATGTAATGCGAG
Grub4.S672	CGGCTGGTGTGATCTTGCGCCGGGCGTCTGTGAAATTGTAGCATTAATGTAATGCGAG
Grub5.S673	CGGCTGGTGTGATCTTGCGCCGGGCGTCTGTGAAATTGTAGCATTAATGTAATGCGAG
Grub7.S692	CGGCTGGTGTGATCTTGCGCCGGGCGTCTGTGAAATTGTAGCATTAATGTAATGCGAG

	470 480 490 500 510 520
Polyph.decem.S675	ACCAAGCGCGTGCGACGGTCGTCGTGTCATAGATTATGCATTACGACGTCAGACCG
Polyph.decem.S685	ACCAAGCGCGTGCGACGGTCGTCGTGTCATAGATTATGCATTACGACGTCAGACCG
Polyph.barbat.S674	ACCAAGTGCGTGCGACGGTCGTCGTGTCATAGATTATGCATTACGACGTCAGACCG
Grub1.S669	ACCAAGTGCGTGCGACGGTCGTCGTGTCATAGATTATGCATTACGACGTCAGACCG
Grub3.S671	ACCAAGTGCGTGCGACGGTCGTCGTGTCATAGATTATGCATTACGACGTCAGACCG
Grub4.S672	ACCAAGTGCGTGCGACGGTCGTCGTGTCATAGATTATGCATTACGACGTCAGACCG
Grub5.S673	ACCAAGTGCGTGCGACGGTCGTCGTGTCATAGATTATGCATTACGACGTCAGACCG
Grub6.S692	ACCAAGTGCGTGCGACGGTCGTCGTGTCATAGATTATGCATTACGACGTCAGACCG

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                                 530       540       550       560       570
                                   .         .         .         .         .
Polyph.decem.S675   TTCGCAATACGCGGTCCGCCGGAATGCGTGGCACGCGTCAAGCGAAAACGCGACCT
Polyph.decem.S685   TTCGCAATACGCGGTCCGCCGGAATGCGTGGCACGCGTCAAGCGAAAACGCGACCT
Polyph.barbat.S674  TTCGCAATACGCGGTCCGCCGGAATGCGTGGCACGCGTCAAGCGAAAACGCGACCT
Grub1.S669          TTCGCAATACGCGGTCCGCCGGAATGCGTGGCACGCGTCAAGCGAAAACGCGACCT
Grub3S671           TTCGCAATACGCGGTCCGCCGGAATGCGTGGCACGCGTCAAGCGAAAACGCGACCT
Grub4.S671          TTCGCAATACGCGGTCCGCCGGAATGCGTGGCACGCGTCAAGCGAAAACGCGACCT
Grub5.S673          TTCGCAATACGCGGTCCGCCGGAATGCGTGGCACGCGTCAAGCGAAAACGCGACCT
Grub6.S692          TTCGCAATACGCGGTCCGCCGGAATGCGTGGCACGCGTCAAGCGAAAACGCGACCT
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